

Scrapping treaties is wrong

The United States seems to be losing patience with the only existing agreement on strategic arms. But the US's tetchy demeanour may prove to be an embarrassment.

PRESIDENT Ronald Reagan's announcement last week that the United States will not necessarily honour the Salt II treaty after September of this year is almost certain to prove more of an embarrassment for the United States than for the Soviet Union. While it is good news that the president has agreed to scrap two Poseidon submarines to make room, within the limits of Salt II, for a new Trident submarine in the US strategic fleet, it is bad news that he has also, for practical purposes, set an arbitrary limit on the time for which the complicated negotiations at Geneva should continue while simultaneously diminishing the chance of their success. The immediate consequence is that the other members of the North Atlantic Treaty Organization (whose foreign ministers were conveniently meeting last week in Canada) have been outspokenly dismayed by what seems to have been a needless provocation in the fragile climate of the arms control process. Much more of this and Europe's inclination towards Gallic ways will be strengthened. Worse still, the United States may find that it has lost the benefit of the charitable doubt it has enjoyed for the past six years. All kinds of people, many in the United States, will now be asking whether the administration can be serious in its protestations that arms control is at the centre of its attention.

The most curious feature of the president's decision is that it appears to have been unprovoked. This is not the first time that a working strategic submarine has had to be laid up so as not to violate Salt II; the same happened last September. Ostensibly, the warning that Salt II may not be kept after September is intended as a riposte to alleged Soviet violations of the treaty, and indeed is conditional on the failure of the Soviet government to mend its ways, yet nothing new has happened in the past few months except that Mr Mikhail Gorbachev has put out a whole string of proposals on arms control, many of which are sensible and well worth talking about.

Change

It is true that much has changed since Salt II was signed (it has not been ratified) just ten years ago. The Soviet Union has broken the spirit but not the letter of the treaty by deploying several hundred SS20 missiles in positions from which targets in Western Europe could be attacked, and has been able to modernize its force of longer range missiles by pretending that the new SS25 missiles are but more modern versions of older rockets. And, in an undisputed violation of the treaty, the Soviet Union has also taken to coding the telemetry from test rockets, thus depriving the United States of one of the most effective means at its disposal of "national verification", acknowledged by the treaty to be necessary. But there is no suggestion that the Soviet Union has broken the chief restriction of its freedom of action — the numerical ceiling on the missiles it may deploy.

Nothing in this list of supposed transgressions justifies the US administration's display of impatience last week. The most substantial complaint, about the encryption of telemetry from Soviet test rockets, is long-standing. Is President Reagan now saying that the United States will withdraw its threat to breach Salt II if Soviet telemetry is no longer encrypted? Or is he asking that the SS25s should be dismantled, which is a much taller and

more impractical order, and which would also raise questions from the Soviet Union about the legitimacy, within Salt II, of the development of both the MX and the Midgetman missiles in the United States? Even if last week's statement were intended merely as a piece of public diplomacy, it has the thoroughly unsatisfactory feature that neither the Soviet Union nor the rest of the world can tell what must be done to ensure that the United States does not follow the threatened breach of Salt II.

All these are matters that are capable of being resolved within the framework of the bilateral negotiations of Geneva. Indeed, to the extent that one of the chief objectives of those negotiations is an agreement on strategic weapons at a reduced level (half as many warheads as at present, or thereabouts), the negotiations are plainly also a way of replacing Salt II by a treaty that is not "fatally flawed" — President Reagan's description last week which is ominously borrowed from his rhetoric of the 1980 election campaign. One obvious defect is that it counts missiles rather than warheads (although the calculations of supposed strategic balance are based on the assumption that missiles with multiple warheads are fitted with their full complement). The treaty is also cumbersome, well provided with potential bones of contention. If the United States were now urging the potential benefits of a better treaty, nobody would complain. From the Soviet point of view, there are also good reasons why Salt II is unsatisfactory. It says nothing about the British and French nuclear forces, for example, although the calculations of strategic balance were made by subtracting from the US missile quota an allowance for the European striking forces of a decade ago. Both sides could fairly ask for a better treaty than Salt II. But Salt II is far better than no treaty at all. Moreover, there is no sense in which the present treaty, which allows each side the equivalent of 5,000 nuclear warheads, restricts the freedom to deploy a sufficient retaliatory force.

These are the reasons why last week's announcement that Salt II will not necessarily be considered binding later in the year is bound to unsettle opinion and policy elsewhere. Both parties to the Geneva negotiations have been saying for months that the other is not seriously interested in an agreement, but Mr Mikhail Gorbachev is the one who has been stunning everybody with a string of tangible proposals, some of which may be practical. The US administration seems not to have calculated (or cared?) what the effect will be in, say, Western Europe of presenting itself as the party that will tear up an existing treaty unless the other meets certain vague requirements. The new development seems all the more sombre because it virtually coincides with the curious refusal of the United States at Berne to sign the modest but useful agreement (within the Helsinki treaty framework) on personal and professional freedom to travel. One common reaction will be to wonder whether the time has come for separate agreement with the Soviet Union on the British and French nuclear forces, which would disastrously spell the end of thirty years of planning for the defence of Western Europe. Another, more constructive, will be to ask whether the cause of arms control is not so important that it can no longer be left to the superpowers. One specific issue is likely to become a thorn in Mr Reagan's flesh in the next few months; hitherto, most European



governments have accepted that the time has not been ripe for a comprehensive test-ban treaty, given the US argument that there is a need to develop warheads for the Midgetman, but that position will now almost certainly be eroded.

Where all this will end is anybody's guess. The United States seems to have calculated that, what with one thing and another, its impatience will not dissuade Mr Gorbachev from going to Washington later in the year for a replay of last year's Geneva summit. The calculations may be correct, but the consequences will be that Mr Gorbachev will travel intransigently. But he may decide not to go at all, which will be a greater embarrassment for the United States in its dealings with its friends. □

Who pays for AIDS?

The alarming financial implications of the US epidemic of AIDS creates another social dilemma

ACQUIRED immune deficiency syndrome (AIDS), a haunting source of trouble for the past five years, has become a problem for the US insurance industry. In several states, it is now a matter for vigorous controversy whether insurance companies should be allowed to deny health or life insurance to people who test positively for antibodies against the virus for AIDS, human immunodeficiency virus (HIV). California and Wisconsin have already enacted legislation that forbids the use of HIV antibody tests in this way, and similar proposals are being considered in New York. In the District of Columbia, the council is likely soon to approve a bill that would forbid insurance companies using any type of test for AIDS for the purpose of determining insurability. The insurance industry, aware that similar proposals are likely to be enacted elsewhere, has started an energetic (and expensive) lobbying campaign to oppose legislation of this kind.

The kernel of the problem is that a positive result in a test for HIV antibodies by no means proves that a person will develop AIDS, which in its fully developed form is at present always fatal. Estimates of the proportion of those who test positively who will eventually succumb to the disease vary, and because of the long incubation period of the virus it will be years before this is known with any accuracy. The federal Centers for Disease Control estimate that the proportion is between 5 and 19 per cent, although it could be much higher. As a consequence, and because the hospital costs of an AIDS patient average \$147,000, those who test positively are in practice uninsurable.

Recent estimates of the number of people in the United States infected with the virus range from 500,000 to 2 million. The number of AIDS cases may reach 100,000 by 1989 if current trends continue, corresponding to claims under "life" (more accurately, death) insurance policies worth perhaps \$5,000 million by the early 1990s. But if the insurance companies are able to deny insurance to those testing positively to the antibodies, many seeking new insurance in most of the United States are liable to find themselves being denied coverage.

AIDS is not unique; similar problems have been raised by other high-risk conditions such as diabetes and cystic fibrosis. A solution that has been adopted in nine states so far is the "high-risk pool", a special state-administered insurance scheme for individuals known to be bad medical risks. The pool is maintained by contributions from insurance companies in approximate proportion to the amount of business they do. Individual premiums are capped, typically at 150 per cent of the standard premium, ensuring that high-risk individuals can buy coverage.

Organizations representing victims of chronic diseases applaud such schemes, which allow their members to get insured. The insurance industry approves because it avoids divisive strains on individual companies. Ultimately, of course, other insurers still pay for the misfortunes of the high-risk groups. Congress should consider legislation that would effectively oblige other states to set up high-risk pools, bringing them into line with those that already have successful schemes. □

Who will pity Africa?

The plight of Africa commands attention, but throwing money in that direction is no solution.

THIS has been a bad week for Africa, but that is not unusual. The number of black Africans killed in the Republic of South Africa (mostly in intra-racial fighting) exceeds the number of those killed by radiation at Chernobyl. The death-toll among students in Nigeria, where most of the universities have been closed, is almost as great. The willingness of perhaps 20 million people elsewhere to run to raise funds for Africa is a moving proof that Africa can still stir the world's compassion; it is only natural that the organizer, Mr Bob Geldof, should be furious that the special session of the United Nations last week failed to echo his runners' enthusiasm. But Geldof's tirade last week against the "politicians", whom he alleges have neglected a golden opportunity to put Africa to rights, is thoroughly misplaced, at least so long as it stems from the assumption that Africa will become a Garden of Eden if only enough money is thrown at it.

The plain truth is that many of Africa's most serious problems have been created in Africa by Africans, and that only palliation of them is possible while these policies persist. The recent history of Africa is a sufficient proof of the validity of that charge. Ghana, relatively prosperous among African countries a quarter of a century ago on the basis of a successful cocoa-growing industry, is now among the poorest. Nigeria, rich more recently at the height of the oil boom, has squandered its brief wealth, becoming one of the world's major debtors. Uganda, already a kind of paradise in the early 1960s, has now settled for inter-tribal violence and the chaos that follows. Ethiopia, sadly ravaged by two years of serious drought, would have found that ecological catastrophe less damaging if the previous food distribution system had not been undermined by the government's ideological conviction that middle-men (admittedly a grasping lot) should be replaced by a public bureaucracy whose efficiency is notoriously below par, and which offers farmers prices that provide no worthwhile incentive to increase production. This same error is the rule elsewhere in Africa, East and West, where governments' attempts to keep their urban populations happy by fixing food prices at unrealistically low levels has contributed powerfully to the decline of agriculture, the hunger of those same urban populations and the dependence of potentially fertile Africa on food imports. Mr George Shultz, the US Secretary of State, was right to say, at last week's meeting of the United Nations, that poor Africa's governments should prudently mend their ways. Geldof and the many who contributed to international fund-raising should understand that as well.

This does not mean that there is nothing to be done. Africa's request of the United Nations last week was for an increase of aid by donor nations amounting to more than \$80,000 million over the next four years, together with a moratorium on foreign debts that would cost about half as much. Given the need, the figures are not unreasonable. But there was never a chance that the rich countries would or could respond on such a scale.

Indeed, the years immediately ahead are likely to make the rich countries seem to Africans more skinflint than they have ever been, as the United States struggles to control its budget deficit and cuts its direct contributions to foreign aid in the process. Yet there are now enough illustrations of how development projects can succeed, even in Africa, to suggest that many of Africa's problems could be tackled successfully with much more modest sums of money. What is needed now is not a meeting of the United Nations at which politicians rehearse their government's well-known positions, but a practical clearing-house for the good ideas that have already contributed to the improvement of African conditions on a small scale, and which could, with the imagination and enthusiasm of the Geldofs of this world, be spread more widely. □

US research funds

Defense Department to shed unwanted monies

Washington

A FIGHT is brewing in the US Senate over provisions in a money bill to divert \$80 million of defence research funds to support unreviewed research and construction projects at 10 named universities. The Department of Defense (DoD) has refused to allocate funds that Congress has already pressed upon it for the purpose, and support is growing for an amendment that would free the \$80 million for competitively awarded research.

The disputed funds were added piecemeal to DoD's appropriations during the legislative process as rewards for political favours by influential members of Congress. None of the 10 named projects has been subjected to competitive merit review, and the favoured institutions are "not the sort of places where one would normally go" to find the best high-tech research, according to one DoD official.

Academic and scientific organizations are uniformly opposed to Congress awarding research and construction funds by political horse-trading. But most of the disputed projects are in the home states of members of Congress who occupy senior positions on key committees, and the effort to block them, to be introduced in the Senate by John Danforth (Republican, Missouri) will be hard fought.

Ten such "pork barrel" projects worth \$65 million were provided for in DoD's 1986 appropriations bill, which passed Congress at the end of last year. Several of them, notably the \$13.5 million proposal of Northeastern University for a research complex, consist mainly of buildings and look more like campus improvement schemes than defence research.

Defense Secretary Caspar Weinberger refused to spend the \$65 million on the grounds that DoD had no legal authority to support construction projects from its research account, and that the sums were recommended, not mandated, in the bill.

DoD also invited the universities in question to submit research proposals that would be subject to normal competitive review; the reviews are still in progress. But Weinberger has also made plain that DoD would oppose non-competitive construction funding even if it had legal authority to spend the money. "It isn't a matter of legality, it's a matter of policy", says Robert Rozenzweig of the Association of American Universities, which has consistently opposed pork-barrelling.

In response to DoD's refusal, seven US Senators last month introduced wording into an emergency supplemental bill ap-

proved by the Senate Appropriations Committee that would, if approved by Congress, oblige DoD to spend the money anyway. In addition, the committee introduced a special provision that would allocate an extra \$25 million for a proposed Center for Science and Engineering Technologies at Arizona State University. A spokesman for the university explained that the proposal was the consequence of efforts to help the university by Senator Dennis DeConcini (Democrat, Arizona).

A sum of \$10 million that had been intended for a Floating Point Systems supercomputer at Cornell University was dropped after Cornell's president, Frank Rhodes, refused on principle to accept the grant unless it had been awarded by competitive review. Cornell's proposal is now

being scrutinized by DoD; ironically, one DoD source close to the review process says that of all the original pork-barrel proposals, the supercomputer is the most likely to be successful in the review.

Pork-barrel provision have been used in numerous federal appropriations bills over the years to buy special favours for universities, but there is now alarm in the academic community over the unprecedented scale of the proposals in the 1986 DoD appropriation.

One previous attempt to defeat the pork-barrel provisions, led by Senator William Proxmire (Democrat, Wisconsin) was defeated in the appropriations committee. But supporters of Danforth's amendment believe that the bipartisan support now emerging gives it a good chance when it is introduced on the floor of the Senate, probably in the first half of June. Among the supporters of the move is, surprisingly, Senator Barry Goldwater, the Arizona Republican who heads the Senate Armed Services Committee. DoD's stand might carry the day.

Tim Beardsley

Space launchers

Launch failure; boardroom fracas

THE failure of the third cryogenic stage of Ariane, Europe's space launcher, at the weekend, coming after the recent serious setback to the US space programme, has put the West's space launching capacity into jeopardy. It has also thrown into vivid relief the sacking just a couple of days earlier of the president of the company that makes Ariane rocket motors.

Ariane has now lost two out of its last three launches and four out of the past eighteen — the last two as a result of third-stage failure. Last week's launch was aborted with an Intelsat communications satellite aboard. A long investigation by an independent commission of all possible sources of error in the manufacture of the liquid hydrogen-liquid oxygen third stage will now be started, and delays to the programme now seem likely to be months rather than weeks. A complete redesign of the third stage engine might be necessary, so even longer delays are possible.

Strangely enough the president of the company, Roger Lesgards, was precipitately sacked last week in circumstances that suggest that the reasons may have been political rather than commercial.

M. Lesgards had been a close adviser of the previous minister of research and technology, Jean-Pierre Chevènement, and is thought to have been letting his feelings about the recent reductions of research support become widely known. Contracts with the government and government agencies are the chief source of work for SEP (Société Européenne de Propulsion).

Late last week, M. Lesgards had al-

ready quit his office and could not be reached at home, but he has made no secret of his anger at his treatment by the board of SEP. The company appears to be doing well commercially, having increased its turnover by 42 per cent in the year to October 1985 and having taken orders worth FF 3,000 million (£300 million) during last year. With half of the company's activity concerned with Ariane, whose commercial prospects have been strengthened since the shuttle disaster and failures of US Titan and Delta rockets, SEP's future seemed bright.

Lesgards' successor is M. Jean Sollier of the aerospace company SNECMA, another supplier for the Ariane programme. SNECMA happens to hold a marginal majority (50.14 per cent) of the shares of SEP, and there has recently been some friction over the rate and regularity with which SEP has been supplying components for Ariane.

Robert Walgate

• Meanwhile, Britain changed its mind last week about which rocket will launch the first Skynet military communications satellite. The Ministry of Defence can get a guaranteed launch date "several months" earlier by using Ariane, rather than the US space shuttle, to launch Skynet 4B, the first satellite in the series. It has therefore cancelled its booking with the shuttle and Ariane will launch Skynet 4B in late 1987 unless the latest setback calls for more changes to the schedule. The shuttle will still carry the next satellite, Skynet 4A, in 1988, and Ariane will carry Skynet 4C in 1989. □

Chernobyl

Fusion may be a better way

ONE lesson to be learned from last month's nuclear accident is that there should be a renewed effort in thermonuclear fusion. This was the opinion offered last week by Academician Evgenii Velikhov, vice-president of the Soviet academy and a member of the investigating commission at Chernobyl, speaking at a press conference in Moscow.

The same point was echoed at an international conference on nuclear technology in Yalta last week. Academician Boris Kadomtsev, director of plasma physics at the Soviet Atomic Energy Institute, called for greater international collaboration in the field. The Soviet Union is a partner in the International Atomic Energy Agency's project for the design of a thermonuclear reactor and also has a number of bilateral agreements on collaboration on fusion work.

The main emphasis of Velikhov's statement was that the clean-up operation at Chernobyl has presented those involved with unprecedented problems. Little had been done in advance, he said, to construct models that would assist in the management of the damaged reactor, while such data as were available were often contradictory. The mound of concrete, lead and boron under which the damaged Number 4 reactor is now buried prevents access, so that it will be necessary to estimate its future behaviour by extrapolation. Even so, it appears that it has been possible to install sensors for temperature and radiation intensity in and around the reactor.

After the burial of the reactor, the main task has been, according to Velikhov, that of trapping airborne particles, for which purpose a special film of polythene has been used. Velikhov added that, in any case, only a "certain percentage" of the fission products in the reactor core has reached the atmosphere.

As the most urgent problems were dealt with, others emerged. As well as the pollution of agricultural land and livestock, the forests of the Polesye region and their wildlife aroused concern. These forests — mostly managed plantations of conifers (although the original tree-cover was deciduous) — appear to have taken the brunt of the pollution. Valerii Brezhnev, deputy minister for forests of the Ukrainian SSR, said last week that many plantations will have to be felled and the clearings ploughed up, while veterinary monitoring of wild animals (elk, roe deer, boar and other species) has begun.

Boris Paton, chairman of the Ukrainian Academy of Sciences, also said last week that there are plans for establishing "artificial geochemical barriers" on the "migration paths of radionuclides in the

groundwater". The river Pripyat' and the Kiev reservoir were mentioned specifically. Paton was presumably referring to techniques more specific than the emergency mechanical barriers thrown up to prevent the run-off of rainwater from Chernobyl into the river Pripyat', and which were said to have been completed two weeks after the accident.

On the future of nuclear energy in the Soviet Union, there seems to be a conflict of opinion. The first deputy minister for electrification of the Soviet Union, Alexsei Makukhin, said somewhat off-handedly last week that the accident at Chernobyl had been a "hitch" in the "mastering of the atom". Velikhov was more cautious, saying that although it is unlikely that reactors of the Chernobyl type will be closed down, "it is unlikely that they will stay in the same form".

Vera Rich

Counting the score on transplants

Washington

PROFESSOR Robert Gale, normally to be found at the University of California, Los Angeles, but who has spent most of the past month in Moscow carrying out bone marrow transplantations on victims of the Chernobyl accident, has contributed an account of the present status of marrow transplantation to a new journal *Bone Marrow Transplantation*, first published this week. Gale estimates that there will be some 2,500 transplantations worldwide during 1986, not many fewer than the 4,000 such operations reported to the International Bone Marrow Registry during the past decade.

Radiation exposure is a comparatively rare cause for transplantation, which is most commonly used in the treatment of leukaemia and other diseases of the blood-forming process. The international registry is meant as a means by which data about the effectiveness of transplant operations can be compiled and compared.

Gale's article, which is the first in the new journal, cannot fail to be widely read by those elsewhere than in the Soviet Union who are concerned to know what part marrow transplantation should have in case of future nuclear accidents.

The new journal, published by Macmillan from the United Kingdom, is meant to be a quarterly in the first instance. The editor is Dr John Goldman from the Medical Research Council's leukaemia unit at the Royal Postgraduate Medical School, London. Dr Gale is the journal's co-editor for the United States and Canada. □

British universities

Reputation by repute

BRITISH academics learned last week a great deal more about the reasons why their institutions had done relatively well or badly in the carve-up of the public subsidy for the impending academic year (see *Nature* 321, 459; 1986). The explanations are contained in letters sent to the heads of academic institutions by the University Grants Committee (UGC), and together constitute the most detailed survey of the state of British universities ever.

The two successive sheaves of letters are the first steps in UGC's policy of concentrating resources considered to be inadequate on what are deemed to be the institutions of the highest quality. In this first stage, UGC has made relatively modest steps in that direction, but in the autumn a further word-processing exercise will tell universities what they can expect to receive from public funds in the succeeding three academic years (1987–90).

Apart from providing universities individually with a inkling of how the harsher winds will be blowing in the autumn, last week's letters give the university system as a whole warning of far-reaching and unexpected structural changes. In particular, it is now clear that UGC intends to give less on account of the cost of student education to universities (Oxbridge and some others) organized in a collegiate fashion, and that it will in future exercise its right to deal individually with the member institutions of federal universities.

The first change arises because the collegiate universities and the several colleges to which students are required to belong are able to charge separate tuition fees, mostly paid in respect of British students by local education authorities. The universities chiefly concerned are Oxford and Cambridge, but Durham, Kent, Lancaster and York charge similar but smaller fees. By deciding that half the income derived from college fees should be subtracted from the annual recurrent grant, UGC is in the short run releasing funds to spend in universities under greater pressure. With the prospect that the time will come when British students' fees will not automatically be paid by local authorities, UGC is requiring Oxbridge to choose between conformity and the privatization of at least their collegiate functions.

Last week's sheaf of letters also explains why both Oxford and Cambridge were placed below the middle of the previous week's league table, with increases of cash income of zero and 0.7 per cent respectively, compared with the average for the system as a whole of 1.0 per cent.

UGC's decision to circumvent the

federal structure of the University of London by providing its constituent colleges with an account of what the future holds for them (the University of Wales has always been dealt with in this way) is also potentially far-reaching, and could presage the break-up of the university. Imperial College has been able to talk directly to UGC since the early 1960s, but the other member colleges have had to wait on the university court for the reallocation of UGC's grant to the university as a whole. Now that the university has been reorganized into only five general colleges and the number of its medical schools is shrinking by amalgamation, the proof of the case for the continuation of what is widely thought to be an inefficient system will fall to the central organization.

The consequence of last week's letters from UGC which cannot as yet be calculated is the effect of its opinions on student recruitment. All university departments have been categorized on the basis of their research performance as average, below

average and above average, while departments whose research attainment is judged to be of international character are singled out for special mention. UGC has said separately that it will attempt to categorize departments by the quality of their teaching when there are tools available for carrying out that task. Meanwhile, however, it must be expected that students will flock to the departments praised by UGC and will shun the others, which may accelerate the selectivity exercise.

Many academics are complaining that the time remaining between now and the autumn is not sufficient for the implications of UGC's judgement to be grasped. A crucial unknown quantity is whether the new Secretary of State for Education and Science, Kenneth Baker, will be able to wring extra funds from the Treasury. Nothing was heard of Baker last week. □

● The grant for 1986-87 for the University of Essex has not been reduced (as reported last week) by 0.5 per cent but, rather, increased by 1.7 per cent.

Chilean physicians

Campaign against torture grows

Philadelphia

CHILEAN physicians are leading a new national movement demanding an end to the dictatorship of General Augusto Pinochet. The Chilean Medical Association (Colegio Medico de Chile, CMC), which has for the past four years been investigating and exposing physicians' cooperation in torture by the security forces, has been joined by 17 other professional organizations in a new national assembly (Asamblea Nacional de la Civilidad) that is calling for immediate national democratic elections. With the expiration last week of the assembly's 31 May deadline for its demands, CMC is likely soon to be at the centre of an escalating series of strikes.

The president of CMC, Dr Juan Luis González, and its general secretary, Dr Francisco Rivas, were in Philadelphia last week at the annual meeting of the American Association for the Advancement of Science (AAAS) to receive on behalf of CMC the AAAS award for scientific freedom and responsibility. AAAS has been monitoring human rights in Chile since the early 1980s. The award was shared with Dr Victor Paschakis of the United States, who founded the Society for Social Responsibility in Science.

CMC was honoured for its efforts to track down physicians who cooperated in torture of government prisoners during the 1970s. Some 10,000 civilians, including 80 physicians, were murdered by the secret police or disappeared in the period between 1973 and 1978, and the use of torture is said to have been widespread.

CMC represents 14,000 physicians, more than 90 per cent of Chile's total. It

was run by puppet officers appointed by the government until 1982, when Pinochet gave in to demands for election of officers in order to avoid a showdown with the profession. González and Rivas, who head a council of 23 members, were elected on a three-point platform of solidarity in the profession, peaceful protest against the government's health policies, and investigation of physicians who participate in torture.

As a result of the torture investigations, two physicians, Luis Losada and Manfred



Jurgensen, have been expelled from the association. They admitted examining and certifying as healthy a government prisoner, Frederico Alvarez, who had been tortured and who died a few hours later from respiratory insufficiency due to multiple rib fractures. Two other members of the association accepted suspension for a year for examining a blindfolded

prisoner who was being forced to remain standing, and CMC is continuing investigations of five others.

Rivas suspects that between 70 and 80 CMC members have cooperated in government torture, although in most cases firm evidence is lacking. The association conducts its investigations by examining court records (many of the torture deaths have been examined by the judiciary, although usually to no effect) and by interviewing torture victims or their families. In addition, files left over from the period when CMC was a government organization have provided many clues; the outgoing puppet officials apparently made no attempt to destroy evidence that would help the new CMC council.

Rivas stresses that those under investigation are allowed to retain the lawyer of their choice, at CMC's expense if necessary. Penalties are recommended by CMC's ethics committee and are decided finally by the full 23-member council.

After the most brutal period of Pinochet's rule in the 1970s, there was a lull in political repression in Chile during the early 1980s. But, according to Rivas, the reemergence of organized opposition parties and the end of a temporary economic boom has increased discontent, so that repression is once again on the rise. Recently the Reagan administration has backed away from its policy of "quiet diplomacy" towards Chile, and spokesmen have started to refer to the administration's opposition to dictatorships both of the left and the right.

CMC has already held one and two-day strikes of its members to protest against the government's summary dismissal of the head of Santiago Medical College, Dr Ricardo Vaccare. According to Rivas, a neurosurgeon in private practice whose work for CMC is unpaid, 85 per cent of members supported the strikes. It is therefore not surprising that CMC's activities have attracted the government's unfavourable attentions. Rivas says that CMC officials frequently receive anonymous death threats to themselves and their families and are often shadowed by secret police. In recent months CMC's headquarters in Santiago has been under constant overt police surveillance.

Among the organizations supporting CMC in calling for a return to democratic rule is the National Association of Academics of Chilean Universities. CMC has received expressions of moral support from most major national medical associations, although representatives of governmental organizations such as the World Health Organization have declined to offer their support for fear of compromising their impartiality. But the medical profession has already become a significant political force in Chile. Supported or not, physicians seem determined to make a still larger impact.

Tim Beardsley

SDI

Congress embraces restraint

Washington

NEARLY half of the US Senate has signed a letter criticizing the rapid increase of the budget sought by the Reagan administration for the Strategic Defense Initiative (SDI). The administration is asking for a 77 per cent increase in SDI funds for 1987.

The letter was addressed to Senator Barry Goldwater (Republican, Arizona), chairman of the armed services committee, which will be taking up the SDI budget request this month. The 35 Democrats and 9 Republicans say they are concerned that SDI has received "excessive and inappropriate emphasis" in the Department of Defense (DoD) budget request, threatening "vital" military research programmes.

While expressing support for "vigorous" ballistic missile defence research, the senators worry that the SDI research programme is being rushed in an attempt to meet unrealistic deadlines. The administration hopes that a decision about SDI deployment will be made in the early 1990s.

Support for SDI this year is set at \$3,050 million, of which \$2,760 million comes from DoD and the rest from the Department of Energy (DoE). For next year, the administration is seeking \$5,400 million for SDI — \$4,800 million to go to DoD and \$600 million to DoE. The senators argue that SDI funds have out-paced technological progress, and that a 3 per cent increase, allowing for inflation, will keep the programme going on an even keel.

The House of Representatives may take an even more restrictive approach to the SDI budget. Representative Charles Bennett (Democrat, Florida), a member of the House Armed Services Committee, believes there is backing for a freeze at the present year's level of support. This committee will also be considering the SDI appropriations request this month.

The White House has reacted quickly to this rebuke from the Senate. The President's spokesman Larry Speakes said the administration "disagrees strongly" with the letter's recommendation, and urged Congress to support the President's budget request.

In future, the administration should have a new ally in its battle for support of SDI. The Science and Engineering Committee for a Secure World, comprised of some 77 scientists and engineers, believes SDI deserves the "full support of the scientific community, Congress and the American people". Fred Seitz, president emeritus of Rockefeller University and acting chairman of the committee, says the group was formed in response to recent efforts by academics opposed to SDI (see *Nature* 321, 369; 1986). Spokesman John Kwapisz says that one of the committee's chief goals is to correct the growing public misconception that all scientists and engineers oppose SDI. But with no financial support and no clear agenda for the future, Seitz says he cannot tell how large a part the committee will play in the debate over SDI.

Joseph Palca

Laser cut down to size

LOCAL residents in the predominantly rural and conservative area of California in which the Lawrence Livermore Laboratory is located have forced the laboratory to modify its plans to build a free-electron laser (FEL) too big to fit within the laboratory fence. A high-level panel under Dr George Dacey, president of the Sandia National Laboratory, has been convened to settle the issue.

The Livermore FEL is part of the development of the Strategic Defense Initiative (SDI) and in its original form would have cost \$1,000 million. The device was to have been a 300 MeV induction linac (linear accelerator) designed to produce 1 μ m radiation at a power of 100 MW.

Local opposition to the proposal to extend the laboratory's perimeter fence to accommodate such a large machine has led Livermore to propose a new plan for two FELs operating at half the linac voltage (150 MeV), one at the laboratory and one at the army range at White Sands, New Mexico. The second FEL would be

capable of being upgraded to 300 MeV.

Enthusiasm at Livermore for free-electron lasers as part of the SDI programme stems from the recent success of the 1.5 MeV induction linac at Livermore, at which 40 per cent of the beam energy is converted into 3 cm radiation. The larger versions would incorporate long tapering "wiggler" sections for generating radiation output by the motion of fast electrons through a corrugated magnetic field.

Among the outstanding problems are the construction of a pair of laser mirrors separated by 4 km so as to match the linac beam. Another problem faces the "second" FEL — apparently neither Livermore nor the US Army will accept responsibility for its maintenance.

Livermore is now the main centre in the United States for military FEL research, with a budget of \$80 million a year. As with many other SDI elements, supporters argue that recent developments constitute a major breakthrough; opponents of the ground-based FEL system say it would be, at best, cumbersome and vulnerable. □

Neolithic Jericho

Ancient wall theory teeters

Rehovoth

HEBREW University Professor Ofar Bar-Yosef seems bent on seeing the walls of Jericho come tumbling down again. Bar-Yosef says that the structure identified by the British archaeologist, the late Dame Kathleen Kenyon, as part of a mediaeval city wall, was in fact part of a flood control system used by farmers to control the flow of water from neighbouring wadis. If confirmed, this may mean that Jericho was not the world's first walled city.

This development has emerged from a programme of research that has thrown new light on the first use by farmers of cereals in the Middle East. Bar-Yosef has, for example, excavated from the site of another Neolithic village at the brief settlement of Netiv Hagdud, 15 km to the north of Jericho, samples of carbonized grain which, according to Mordechai Kislev, contain both wild and cultivated barley dating from the period 8,200 BC to 7,500 BC.

The fact that both wild and cultivated barley were found at the same site suggests that both were growing there 10,000 years ago. Bar-Yosef believes that his new find is the earliest record of the use of cultivated cereals, and he believes that wheat as well as barley was part of the Neolithic farming pattern. By the biblical era, the use of cereals was sufficiently well established to support the well-known statement in *Deuteronomy* that "Man doth not live by bread alone...".

Several research groups in Israel are involved with the search for the origin of modern cereals. Since the discovery almost exactly eighty years ago of a surviving primordial wild wheat plant growing out of a crevice in a rock near the village of Rosh, in what was then Turkish Palestine, much has been done to conserve these ancient plants. The agronomist Aaron Aaronsohn who found the first plant, followed by others in Syria and trans-Jordan in succeeding years, had hoped to transfer some of the genetic characteristics of the wild wheat to the commercial strains, but breeding techniques at the time were not good enough. But now Professor Moshe Feldman at the Weizmann Institute, by manipulating chromosomal segments, claims to have incorporated some of the ancient genes into commercial strains of wheat that yield as much as existing varieties but boast a protein content up to six per cent greater.

Meanwhile, Feldman and his colleagues are asking that the existing fields of wild wheat will be carefully fenced off so as to preserve the pristine genetic constitution of the plants.

Nechemia Meyers

Japanese psychiatry

Fetus research criticized

Tokyo

ARGUMENT over the rights of psychiatric patients in Japan has resurfaced with the release of a report from the Japanese Society of Psychiatry and Neurology's human rights committee. The report censures the conduct of an experiment on a fetus aborted from a schizophrenic woman. The "human experiment", performed at Gifu University, is seen as a test case by those critical of current psychiatric practice, as is spelt out in a letter from one of their representatives in this issue of (see page 556).

There is general agreement only on the bare facts of the Gifu case. The patient concerned was a 34-year-old schizophrenic woman with a long history of mental illness. She was admitted to a Gifu hospital in January 1984 and found to be about five months pregnant. Shortly afterwards she was transferred to Gifu University Hospital where the pregnancy was terminated. The fetus was dissected and its brain examined for the effect of the drugs the woman had been taking.

It was not until May 1985 that the case attracted the attention of the press. It was alleged that the woman had been forced to undergo an abortion simply so that the doctors could experiment on the fetus. Those critical of psychiatric conduct quickly labelled the case as an example of the "research supremism" that leads psychiatrists to view patients merely as opportunities to further their academic careers. Protests over the case grew to such an extent that fear of violence led the Japanese Society for Biological Psychiatry to cancel a meeting that was to have been held at Gifu with doctors involved in the experiment present.

Two invited foreign speakers also had to abandon their talks even though they were moved to a secret venue. Both speakers have expressed their views in *Nature* (319, 172; 1986; and 320, 392; 1986). They had been told that protesters were radical "anti-psychiatrists" who frequently disrupted meetings with violence and that Dr Moriyama of Tokyo University who was their *de facto* leader. Dr Moriyama has clearly rejected the label of "anti-psychiatrist" for himself and denied any violent intent on the part of Gifu protesters (*Nature* 318, 308; 1986).

Did the Gifu case involve malpractice? On the evidence supplied by the Gifu doctors alone there is no reason to think so. They say that the woman was admitted to hospital because she was showing schizophrenic symptoms; that she freely chose to have an abortion because of the difficulties she would face in bringing up a child; and that the idea of examining the fetus came up only after the woman had

decided to have an abortion.

The Society of Psychiatry and Neurology's report does not accept this view of events and raises some fresh ethical issues. In the committee's view there was evidence that the woman wished to have the baby and that she had been pushed into receiving an abortion: "informed consent" had not been properly obtained. There were also suspicions that admission procedures had not been properly observed. Finally, consent to the fetal experiment was not obtained from the patient, only from her mother.

Further problems stem from the experiment. Japan is without clear guidelines for such experiments so criticism was made in the light of foreign guidelines, principally there of Britain's Department of Health. Here it is made clear that a living fetus retains its rights even if it is going to die in an abortion — thus a pregnant patient should not be allowed to take a drug that could harm the fetus, even if an abortion has already been planned and even if the

doctors would like to know what the effect of the drug on the fetus would be. It is not clear whether the Gifu doctors agree with this point, and further public discussion of the issues involved is called for in the report.

What is certain is that there is a need in Japan for clear research guidelines for those who perform experiments involving human beings. At least reform is now in the air. The legal rights of psychiatric patients (or the lack of them) has already become a topic of international concern and the Ministry of Health and Welfare has promised to produce changes in the law by next spring. Ethical committees are now appearing in hospitals and increasing numbers of doctors are involved in providing alternatives, such as community care, to the long stays in isolated mental hospitals that are a much criticized feature of the Japanese psychiatric system. Soon, the present controversy may be seen as one step towards an improved psychiatric care system.

Alun Anderson

Nature in Tokyo: next week (9 June) David Swinbanks takes over from Alun Anderson as *Nature's* Tokyo Correspondent.

Ageing research

Japan faces the old old problem

Tokyo

A NEW study from Japan's Council for Science and Technology tackles the nation's most serious long-term problem — its rapidly ageing population.

At present Japan has relatively few people over 65 compared with those in the working population; European countries and the United States have to divert a far larger percentage of their economic resources to care of the aged. But in 15 years' time the situation will have reversed. By the year 2000, one out of every five Japanese will be more than 60 years old. And by 2025 there will be two and a half times as many elderly people per worker as at present. The trend is a result of the remarkable longevity achieved by the Japanese coupled with a declining birth rate.

The government is already planning changes in health insurance and pension laws to ensure that it is not saddled with a

bill it cannot pay in the next century. The proposals from the Council for Science and Technology are complimentary changes in the scientific field and are expected to serve as guidelines for the next ten years. The council, directly answerable to the prime minister, is the top government policy body and all government ministries are expected to begin promoting research according to the guidelines. A likely outcome is the establishment of a new research centre for the study of ageing, perhaps equivalent to the US National Institute of Aging.

Senile dementia is likely to be the main research topic. In Japan around 5 per cent of the over-65 age group (more than 600,000 people) suffer from senile dementia, causing serious social problems. This total will double by the end of the century. Some means, the report says, must be found to enable old people to live healthy and independent lives to maintain the national economy. Some forms of senile dementia are largely due to cerebrovascular disorders and there have already been successes in treatment. Research will focus on finding methods of early diagnosis when chances of recovery are high. The causes of other forms of senile dementia are still unknown. Alzheimer's disease, already a major problem in advanced countries with large populations of the elderly, will be another major research topic, as will improved methods of nursing.

Alun Anderson



Yellow rain

Another treaty threatened

Washington

THE Canadian government last week reluctantly released new information casting further doubt on charges that the Soviet Union has engaged in biological warfare in South-East Asia. The new data show that trichothecene toxins, claimed to be the toxic agent in yellow rain, can be found in individuals who do not report being exposed to yellow rain attacks.

The data were collected as part of a Canadian effort to monitor the use of biological and chemical weapons. A spokesman for the Canadian Ministry of External Affairs said investigators obtained 272 blood samples in 1984 from different locations in Thailand. By early summer 1985, Agriculture Canada had analysed all the samples using gas chromatography and mass spectrometry. Blood from five individuals in three different locations showed signs of trichothecene toxins T-2 and HT-2 ranging from 2 to 76 parts in 10^9 . The analysis also correctly detected 7 of 8 control blood samples that had been spiked with 100 parts of 10^9 of trichothecene toxins.

Although the Canadian government has had the results of the analysis for more than a year, government officials are quick to deny that the information was being suppressed. Ron Cleminson for the Ministry of External Affairs explained that the information is for a report to the United Nations on arms-control verification procedures, and was never intended as a study on toxin poisoning.

Officials from the US State Department had no specific comment on the Canadian data. But department official Tom Reich says that the United States still maintains that the Soviet Union engaged in biological warfare in South-East Asia in the early 1980s. Unofficially, however, a government official did say that the new data should have a profound effect on the US position.

The past few weeks have seen a spate of new data on yellow rain. Reports from both Canada and the United Kingdom found insignificant levels of trichothecene toxins in yellow rain samples (*Nature* 320, 699; 1986 and 321, 459; 1986) gathered in 1982. Matthew Meselson of Harvard University, a principal author of the theory that yellow rain is not a biological weapon but rather mass bee defecation, believes that the Canadian evidence makes the US position untenable.

The latest controversy comes as the United States is preparing to attend the Geneva conference reviewing the 1972 Biological Weapons Convention. Hailed as the first step on the road to true disarmament, the treaty requires signatories to destroy existing stockpiles of biological

weapons. It further prohibits any future development of production of biological agents that have no justification for "prophylactic, protective or other peaceful purposes".

The United States now takes the position that the Soviet Union has violated the terms of that treaty. In addition to charges that the Soviet Union has used biological weapons in South-East Asia and Afghanistan, the US government also believes that the Soviets have a biological weapons

French research

Chevènement's expected outburst

JEAN-Pierre Chevènement, the architect of the past administration's buoyant policy on research, last week accused France's new government of "unilateral disarmament in the economic war". He was referring to the cuts on science spending which flow from the conviction of the right-wing Prime Minister, M. Jacques Chirac, that reduced public spending will revitalize the economy by releasing private enterprise from tax burdens.

Chevènement's complaint, published in *Le Monde* last week, stems from his belief that world competition now threatens "whole economic and social systems" and that research is particularly important, and will "more and more decide the winners". So, says Chevènement, the Chirac government's transformation of real growth of four per cent a year in science spending into a four per cent cut was "a grave blow".

Chevènement fears that the new government is allowing the "two conservatisms" against which he had to battle to reestablish themselves. One conservative influence is industry, where only 100 companies employ more than 50 researchers and only 1,500 have any at all. The other is the academic community, which Chevènement fears may abandon its flirtation with industry and retreat to its old ivory towers.

Chevènement has a kind word for General de Gaulle, who he says was the only president before Mitterrand to recognize the importance of research, but who took ten years (1958–68) to make an economic impact with it. But the Mitterrand–Chevènement policies have now been "halted in mid-stream", while power has now returned to those who, under President Pompidou, presided over a steady decline of scientific output. Chevènement quotes Pompidou as saying that, of three ways to lose money "the most agreeable is women, the most rapid gambling, and the most certain research".

Chevènement also credits the new mini-

programme under way at a research facility near Sverdlovsk. But James Leonard, former US disarmament negotiator who negotiated the 1972 treaty, says the United States has made charges that "cannot be substantiated." To reverse the present climate of mistrust, Leonard recommends steps to encourage communication among the superpowers, such as an international registry of high-containment laboratories and greater participation in international scientific exchanges. While there is no indication that the treaty will collapse, Leonard believes the prospects for strengthening or expanding it in the fall review are nil.

Joseph Palca

ster of research and higher education, Alain Devaquet, for "courageously defending" the research councils against dismemberment. On the break-up of his own ministry of research and technology, Chevènement said that it would not now be possible to establish joint programmes across research institutions. What Chirac has dismembered is the "ministry of the future".

Robert Walgate

Optical computer in sight

Edinburgh

A GALAXY of stars of European science politicians gathered at Herriott-Watt University in Edinburgh on Monday to celebrate what former French science minister Pierre Aigrain described as a "spectacular" success along the road to the development of an entire optical computer.

EJOB, the European Joint Optical Bi-stability programme, has cost just 1.8 million ECU (about £1.2 million) over two years but it has stimulated 20 research groups around Europe to produce what was first demonstrated in Edinburgh this week — broad-band optical switching and a basic "classical finite state machine" (the guts of a computing engine) using nothing but lasers and nonlinear optical material. "Massively parallel" optical image processing and new forms of laser-driven television are also just plausible.

Professor Desmond Smith of Herriott-Watt University and leader of the EJOB project, says the programme could absorb a tenfold increase of funding to 10 million ECU per year, but the European Council of Ministers, meeting in Luxembourg next Tuesday, has yet to be convinced of the need for an increase. Parliamentarians at Edinburgh agreed, and pressure is now increasing on European ministers, to divert a small part of the enormous Commission agricultural policy funding to science and technology. Robert Walgate

Beckman anniversary

Analysing profitable research

Fullerton, California

At the age of 86, Arnold O. Beckman still has his sights set on the future.

Beckman launched his corporate career with the innovation of the pH meter in 1935. Many years and many millions of dollars later, the founder and chairman of Beckman Instruments still considers himself more a scientist than a businessman. Today, with his \$1,000 million company safely tucked under the Smith Kline Beckman wing, the former chemistry professor from the California Institute of Technology spends most of his time and money supporting programmes that he hopes will shape scientific enterprise in the years to come. He has contributed more than \$100 million in the past decade, nearly \$70 million in the past year alone.

Last month, at the festivities to mark the company's 50th anniversary, Beckman spelled out his formula for keeping science in the United States alive and well. The key ingredient, he says, is education.

Of the US education system, Beckman says "we've got a big repair job to do". He sees education as a dynamic, rejuvenating escort that directs an individual throughout his or her lifetime, and he feels that the present structure of higher education misrepresents that vision. "This strange idea of dividing education into four-year packages", he says, blurs the mission of college education, which is "to teach people how to learn".

Beckman maintains that degrees awarded at the conclusion of an arbitrary period of time give the impression that education is a static commodity. The one big advantage to be gained from having a PhD, he claims, "is to realize it doesn't mean anything. I've seen PhDs who are nitwits."

How would he restructure higher education? "Like a department store" that people would patronize whenever the need for a particular item arose, not like a remote citadel whose doors shut firmly behind those who graduate.

Certainly Beckman's own career has been spurred on by insatiable curiosity. A blacksmith's son from Cullom, Illinois, he had built his own chemistry laboratory before he reached his teens. He graduated first in high school class and, within four years, earned both bachelor's and master's degrees in chemistry from the University of Illinois. A brief spell at Bell Laboratories gave Beckman the electronics background he would later exploit in instrumentation design. In 1928, when Beckman received his PhD in photochemistry from California Institute of Technology, the institute invited him to join the faculty.

Beckman and his wife Mabel were settled

at Caltech when, late in 1934, one of Beckman's fruit-growing friends asked him to build a device to measure the acidity of lemon juice. Within a few months, Beckman had developed the "acidimeter"; the term pH had not yet been established.

By 1939, Beckman's instrument had spawned a business that demanded a full-time manager. Beckman left Caltech, not without considerable deliberation, to pour his energy into National Technical Laboratories, Inc., which eventually became Beckman Instruments. A year later, his company began marketing the model DU quartz photoelectric spectrophotometer, the Helipot potentiometer, and a portable oxygen analyser. These three products guaranteed the company's success, and by 1966, Beckman had received a business statesman award from Harvard Business School of Southern California.

Beckman's philosophy on research echoes his personal experience. "The end result of research is to find out something useful", he says. "The days when applied science was a step lower than basic science

his executive responsibilities, he has been kept busy practising what he preaches. Last month Caltech students, faculty and trustees gathered for the dedication of a \$6.5 million chemistry building established through a Beckman donation. Beckman had previously remembered his alma mater with \$7 million for an auditorium and behavioural biology laboratory. A \$3.5 million gift created the Beckman Laser Institute and Medical Clinic at the University of California, Irvine and another \$3.5 million set up a vision centre at the University of California, San Francisco.

Beckman also donated \$10 million to form the City of Hope Beckman Research Institute; Stanford University received \$12 million to erect the Arnold and Mabel Beckman Laboratories of Biochemistry. Finally, Beckman outdid himself last October with a \$40 million gift to the University of Illinois, the largest amount ever given by any individual to a public university (see *Nature* 317, 568; 1985).

Although Beckman suggests that he is "not a generous man", this lack of generosity has saddled him, to his dismay, with the label of philanthropist. Today Beckman distributes his beneficence through the Beckman Foundation, which has just two employees, Beckman and his

secretary, and which receives 1,300 proposals every year.

Despite Beckman's apparent nonchalance about his benefactions, he shrewdly manipulates his wealth to promulgate his philosophy. He supports only proposals that fit his criteria for the successful research community. Beckman encourages interdisciplinary interaction, which he thinks will become imperative as science becomes more complex. He also looks for a steady "infusion of young brains, people who don't know what you can't do".

Tempering his confidence in the saving powers of science, Beckman is also keenly aware of the thorny problems it thrusts upon society. Last November, Beckman gave the National Academy of Sciences and the National Academy of Engineering \$20 million for a west coast headquarters to study and adjudicate the ethical and social challenges posed by scientific advance (see *Nature* 318, 97; 1985).

Vital to Beckman's action-oriented formula, however, is one component that cannot easily be institutionalized: enthusiasm, which he considers the best motive for any endeavour. "If you're not enthusiastic about what you're doing, you're probably doing the wrong thing", says the spirited octogenarian. By that measure, Arnold Beckman must be doing all the right things.

Karen Wright



Arnold Beckman at his company's anniversary celebration.

are gone." For Beckman, academic research should not be spiked with financial incentives from outside an institution. Unless patent proceeds go to the individual's employer, a researcher will find himself "working for two masters".

Patent royalties aside, Beckman expresses concern over the lack of reliable sources of research support. "We've been too affluent", he laments. "The money (for research) comes from the public, but look at what the public is interested in; look at the salaries they pay someone for throwing a few baseballs over a plate. I'd like to see a small percentage of the GNP automatically devoted to basic research." Beckman also advocates industry support in the form of cooperative arrangements. These are in industry's best interests, he contends, because industry needs academic links.

Since the 1982 merger with Smith Kline Corporation relieved Beckman of many of

State of Japanese psychiatry

SIR—I have read the letters from T.J. Crow (*Nature* 319, 172; 1986) and D.P. van Kammen (*Nature* 320, 392; 1986) and greatly appreciate the interest shown by both authors in my letter on the deplorable situation in Japan's psychiatric hospitals (*Nature* 318, 308; 1986). I should like to repeat once again that the protest action taken against the seventh congress of the Japanese Society for Biological Psychiatry was not directed against either of these visiting scientists.

The reason for the protest was that, in Japan, the scientists responsible for experiments involving humans often refuse to acknowledge criticism. As they remain silent and avoid discussion, concerned people have to resort to protest. Such action is then sensationalized and described as "violent". Protest actions are not illegal and it is regrettable that this is their only response.

This year, on 27 March, we were able to carry out a discussion in the assembly hall of the eighth congress on biological psychiatry held in Kanazawa. We discussed with many members of the society the problem of the previously mentioned experiment on a human fetus carried out by Dr Namba and his colleagues. The doctors actually involved in the experiment did not participate in the discussion. Dr N. Hatotani, the chairman of the board of directors of the society, answered written enquiries supplied beforehand and commented that the experiment of Dr Namba was not to be censured so much. However, the discussion made clear that the investigation carried out by the society into Dr Namba's experiment was inadequate. The society had heard about the experiment from Dr Namba but did not listen to the views of those who had brought up the ethical problems of the experiment. We suggested this was biased and criticized such a method of investigation. Dr Hatotani promised further investigation and discussion.

It seems to us that the measure taken by the society on the ethical problem is quite inadequate. Professor Hidebumi Hazama of Tottori University, a member of the society, has already thrown doubt on the existing ethics of biological psychiatry in Japan and has suggested the need for a system of ethical committees. He had expected the society to take a more positive and serious attitude in this respect (*Seishin igaku* 27, 118–119; 1985).

We also hear that the president of Gifu University orally admonished Dr Namba and other staff in February 1986 about inadequate procedures in an experiment on a human fetus. In the near future, we expect the *ad hoc* committee of the Japanese Society of Psychiatry and Neurology to make a report on that experiment.

I have previously stated that some of the published summaries of papers that were to have been delivered at the 1985 Gifu congress provided evidence of unethical conduct. As requested, we will send these summaries to Dr Crow and Dr van Kammen along with several articles on ethical issues in Japan. In my opinion, in order to assess these papers, it is necessary to be conversant with the real background of such research in Japan, for example the very low standing of patients compared with that of doctors, the difficulty of peer review owing to the absolutism of some professors, the closed character of psychiatric departments to avoid discussion and investigation of their research and so on. Such a situation is far from that envisaged in the *Proposed international guidelines for biomedical research involving human subjects* (CIOMS/WHO, Geneva, 1982).

I sincerely hope that Dr Crow and Dr van Kammen will understand the actual state of Japanese psychiatry. I would advise them both to investigate especially the experiments carried out by Dr Namba and his colleagues and to refer to the more detailed report of the *ad hoc* committee of the Japanese Society of Psychiatry and Neurology which we will send them as soon as possible.

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Dutch legislation

SIR—Your leading articles have frequently highlighted developments in the United States and the United Kingdom concerning ethical and legal aspects of the use of animals in research. New regulations, based on the 1977 Act of Animal Experimentation, recently became effective in the Netherlands.

From 1 January 1986, institutes in which animal experiments are performed require a government licence, issued only if and when a number of conditions are fulfilled. One of these is that the welfare of the experimental animal must be supervised by a specialist in laboratory animal science. This officer, who is on the payroll of the institute, is a veterinarian or biomedical scientist who has taken a one-year postgraduate course in laboratory animal science and who also has some years' experience in this field. He or she reports to the director of the institute and also to the Inspectorate of Veterinary Public Health. If the animals endure extreme and/or unnecessary discomfort, the

inspectorate may advise the Minister of Public Health to withdraw the licence.

A unique provision is that the scientists responsible for animal experiments must have taken an introductory course in laboratory animal science. This applies to all scientists who began animal experimentation after 2 July 1985. Courses in laboratory animal science are now being incorporated into the biomedical education programme of most universities. The course takes three weeks and its contents have to be approved by the Minister of Public Health. It covers topics such as husbandry, gnotobiology and anaesthesiology as well as the ethical aspects of animal experimentation. The primary goal is to set the stage for a justified and humane use of animals for a scientific purpose.

The training programmes are coordinated nationally and commissioned in the department of laboratory animal science at the veterinary faculty in the University of Utrecht.

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Search for truth?

SIR—I disagree with Leo Uzych (*Nature* 320, 480; 1986) that "science involves a search for truth", and with the inference he draws from this, that the orchestration of data in support of a particular point of view is an impediment to scientific advancement. I do not see science as being the gradual excavation of a buried city, but rather as the inspirational formation of structures *de novo*. What one builds is determined by the existing foundations, vision and experimental data. As such, science is not a search for truth but for quality. The extent to which one is justified in advocating individual predilections is therefore a matter for careful judgement based on the consideration that while all constructions are ephemeral, some are more so than others. Unless one is selective with data, all one can hope to produce are bricks for use by others who do not show this restraint.

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Danger of delay for genetic tests

Molecular biology is providing novel means of testing for genetic disease, with the result that researchers are having to spend their time on repetitive testing. Now is the time to make better plans.

THE detractors of molecular biology used to complain that its discoveries might be interesting in an academic fashion, but would have no clinical significance. But recent years have shown that position to be untenable. Indeed, the growing impact of molecular biology on medicine is nurturing a band of clinicians with a deep interest in research while bringing molecular biologists face to face with some of the realities of disease. So much was clear, for example, at the latest International Titisee Conference (24–27 April) on human gene mapping and molecular pathology; progress is rapid, in both the laboratory and the clinic. But it will need a great deal of care, at least in Europe, if present enthusiasms are not to dissipate under the weight of that age-old resentment in which clinicians unthinkingly demand more (and different) diagnostic testing from researchers who believe, rightly or otherwise, that they have better ways in which to spend their time.

The high crest of the common ground between the laboratory and the clinic is undoubtedly, just now, the hunt for the unidentified genes whose defects are responsible for diseases such as cystic fibrosis and muscular dystrophy for which no molecular basis has as yet been determined. The magnitude of this task should not be underestimated, but, as Peter Little explains on page 558, adequate techniques are now available, if not perfected. Each gene has been located in a rather small fragment of a chromosome, but which corresponds to a stretch of DNA which is long by the yardstick of what can now be sequenced. Yet there should not be too long to wait before the first of them is identified.

The molecular probes used in location of these genes are already valuable in the prenatal diagnosis of carriers of defective genes, for the time being only within families in which the disease is known to be present. So long as the genes themselves have not been identified, success depends on a variety of factors — how many independent gene probes there are, how close they lie to the defective gene and whether there are enough close relatives of the two parents of the fetus who are 'informative' in the genetic sense. For some diseases, there is clearly room for improvement, but for many others diagnosis is already reliable. So there is a growing demand, by physicians on behalf of their patients, for tests to be carried

out. Already the demand sometimes outstrips the time and people available, not to mention the people's interest in work that may seem routine.

The procedures are far from simple. Obtaining the prenatal samples is often the least of the problems, but may sometimes be limiting. This may be especially so as pressure grows to replace sampling of the amniotic fluid by sampling of the placenta as a means of obtaining fetal material, which in principle allows diagnosis earlier in pregnancy and thus earlier termination (if indicated). Curiously, although chorionic villus sampling of the placenta was originally thought to be more risky (for the fetus) than amniocentesis, experience has tended to reverse that view, although chorionic villus sampling is still relatively uncommon.

Sampling, however done, is for physicians. It is the samples that demand the immediate attention and the experience of the researchers. For the time being, most of them are likely to be eager to devote time and energy to this task, not only for humanitarian reasons but also, where the still-cryptic genetic diseases are concerned, because of the possibility that any new case may turn out to have a particular genetic defect that provides a short cut to the defective gene itself.

For each disease, this captive enthusiasm of the researchers must be a passing phase. The time must come when they want to move on to other problems. When, for example, the defective gene for cystic fibrosis or muscular dystrophy is identified, the laboratory emphasis is certain to shift to the function of the protein encoded by the gene and to the links between that function and the symptoms of the disease. Because these links are likely to lead both to new discoveries in fundamental science and to new approaches to therapy, it is in everybody's interest that they should be investigated. But who will then take over the presumably increased diagnostic load?

Even if prenatal diagnosis is confined to the industrialized countries, the imagination is challenged by the sheer scale on which testing may be required if the potential of the new discoveries is to be as fully exploited as preventive medicine. Tests which are at present complicated will no doubt be simplified as time goes on, but by then there will be other defective genes for which to test.

The problems occasioned by diseases

that run in families may be relatively small, requiring as they do the testing only of family (including embryonic family) members, most probably at specialist centres which they attend already, so that there is a persuasive argument that the costs of diagnosis and even of the requested termination that may follow will be more than offset by what is saved in there being fewer afflicted patients to treat. But nobody will be surprised if this argument runs into the hidden buffers that so often hinder the application of preventative medicine.

How much greater will be the problems when it becomes feasible and seems to be desirable to screen whole populations for diseases that most often arise as new mutations, not as inherited genetic defects? Or what happens when the several genetic factors contributing to susceptibility to cardiovascular disease have been identified, a question now at the focus of a great deal of research? It should then be possible to categorize groups of people who are at risk, and who could (and should) take preventive steps, but only by complicated population screening.

At the rate at which this science is progressing, the diagnostic needs occasioned even by the simple single-gene diseases are almost certain to outstrip the creation of new testing facilities within public health-care systems, nowhere expansionist. Will medical charities feel compelled to spend less on research and more on testing? Commercial services will certainly grow to meet the need; in the United States, biotechnology companies seem bent on exploiting an emerging market, while, even in Britain, ICI's investment in a centre for providing DNA fingerprinting tests seems well set for moving to meet the growing demand of sophisticated prenatal diagnostic tests.

But where will that leave those who cannot afford the going commercial cost of tests? Especially when the need, in many industrialized communities, is most evident among immigrant populations? It will be frustrating for those laboratory scientists who have helped to open up this new vein of preventative medicine if they are faced, a few years from now, with the unpalatable choice of devoting themselves to routine analysis of a kind that they alone are able to perform or, alternatively, to see the potential benefits of their research simply run to waste.

Peter Newmark

Restriction fragment length polymorphisms

Finding the defective gene

from Peter Little

CHANGES in DNA sequences that introduce or destroy restriction-enzyme cleavage sites and cause restriction fragment length polymorphisms (RFLPs) are the key to identifying the chromosomal location of genes that cause major human genetic diseases. Here I will discuss how useful the linked RFLPs are as a starting point to isolating the defective gene itself, as this remains an unanswered question. I will then describe some ideas about how this might be achieved and some of the technical problems in reaching this goal.

What is the logic behind the use of RFLPs? Once linkage of a single RFLP with a defective gene has been demonstrated, other RFLPs that straddle the affected gene (G) can be identified. These flanking RFLPs (R_L and R_R to the left and right, respectively, of G) can be ordered by observation of the recombination between R_L and R_R , R_L and G or R_R and G. R_L and R_R then define a stretch of DNA that contains the gene. The distance between R_L and R_R must be small if these elements are to be helpful starting points from which to isolate the gene. The proximity with which R_R or R_L can be mapped to G is fixed by the number of meiotic events that are available for the analysis. Lange *et al.*¹ show that if 100 meiotic events could be studied, and assuming that 1 per cent recombination corresponds to a separation of 10^6 base pairs (bp), then the best resolution that could be achieved for the separation of R_R and R_L would be about $2 \times 10^6 \pm 1.4 \times 10^6$ bp of DNA. Steinmetz and co-workers have shown that the relationship between recombination and physical distance in one such locus, H-2 of mouse, is not linear; 1 per cent recombination can be equivalent to 5×10^5 and at worst, from an experimental point of view, 10^7 bp depending on the location within this gene family.²

The DNA probe that defines a RFLP provides indirect information about the physical distance between R_R and R_L , because orthogonal³ or field-inversion⁴ gel electrophoresis can be used to fractionate DNA fragments of up to 2×10^6 bp generated by cutting with rare 6-, 7- or 8-base recognition restriction enzymes in combination with Southern blotting.

The RFLPs define a stretch of DNA which is probably 2×10^6 bp in length and located within or near a specific chromosomal band. Three strategies can be used to isolate the defective gene: candidate gene, chromosome rearrangement and cloning strategies.

The candidate-gene strategy involves the isolation of messenger (m)RNAs

either from genes that are thought to be involved in the genetic defect (for example, those encoding membrane-associated ion transporters in the case of cystic fibrosis); or using randomly selected mRNAs from normal tissues that are affected by the disease, followed either by screening of somatic cell hybrid lines or by *in situ* chromosomal hybridization to show that the mRNA is encoded within the appropriate chromosomal band. Both approaches are technically feasible, but require a formidable amount of work if the normal transcript of the gene is at low abundance.

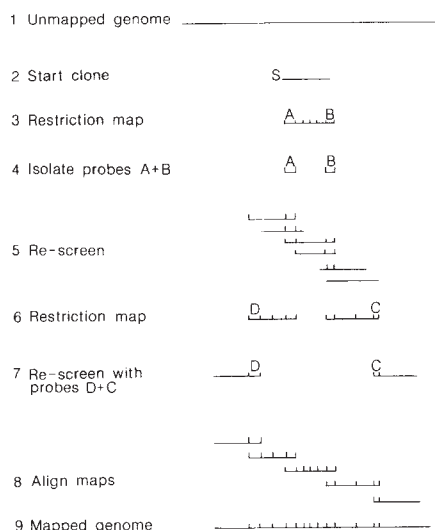


Fig. 1 A and B, C and D; probes for each step.

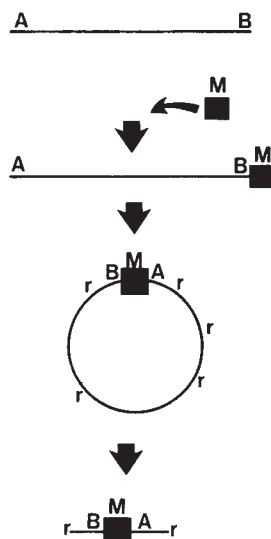


Fig. 2 A and B are sequences 200,000 bp apart. M, marker DNA that can be selected in *E. coli*; r, restriction sites. The jumping library consists of many fragments with r sites at each end containing M and different As and Bs.

The chromosome-rearrangement strategy makes use of chromosome deletions, insertions and translocations to provide a marker that must be close to the affected gene⁵. Where these occur, the markers can be used to order the relative position of genes (or DNA probes) within or near the specific chromosomal region. Very little is known about the relationship of cytologically defined bands and their DNA content, but if one assumes a linear relationship (which is not true of the *Drosophila* polytene chromosome⁶) then a single band should contain about 2×10^6 bp of DNA. There are special circumstances where chromosomal rearrangements of known genes into regions of unknown structure or function can help to identify a region implicated in the gene defect⁷, but these are generally rare and the precision of mapping of rearrangement breakpoints remains close to the level of the single band — again about 2×10^6 bp of DNA. Where they are available, chromosomal rearrangements will be powerful tools for the analysis of specific gene loci. Duchenne muscular dystrophy, Wilms' tumour of the kidney and retinoblastoma are the diseases that appear to be most tractable to this strategy.

The cloning strategy is based on isolating all the DNA between R_L , R_R and, by definition, the gene, and is itself divided into three approaches: mapping, walking and jumping. Mapping was first used by Steinmetz and co-workers^{2,8} who isolated cosmids that contained mouse major histocompatibility complex (MHC) genes by cross-hybridization with MHC class I and II complementary DNA probes. These cosmids were restriction-site mapped and the structure of a substantial portion of the mouse MHC complex was established by aligning cosmids that contained overlapping sequences. The random mapping procedure has been extended by Olson and co-workers (manuscript in preparation) to the yeast genome (10^7 bp) and by Sulston and Olson (in preparation) to the nematode genome (8×10^7 bp, which is about the same size as human chromosome 11). Similar techniques could be applied to a human chromosome isolated from a cell hybrid that only contains the required chromosome. It is well known that cosmids containing human DNA can be isolated from these cell lines by hybridization with human-specific repetitive DNA sequences. The mapping of a whole chromosome is a very considerable challenge but one that is technically achievable within the next 5–10 years. The isolation of cell hybrids that contain only a small region of the human genome by chromosome-mediated gene transfer of a naturally or artificially introduced selectable marker has also been achieved⁹. These cell lines generally contain less than 5×10^6 bp of DNA and could be ideal targets for the

mapping approach.

Walking consists of the systematic isolation of a DNA sequence located at the extreme left or right of a starting (S) cloned DNA and the use of this fragment to screen a library of cloned sequences for DNA that must be adjacent and slightly overlapping with S (ref. 10). This process is called a 'step' — a walk proceeds as a linear sequence of steps. Figure 1 shows the complex set of manipulations comprising a step. At present the cloning vectors with the largest capacity are cosmids that can carry 40,000 bp of human DNA. S.H. Cross and I have developed cosmid vectors that are designed to facilitate this procedure (manuscript in preparation). If the markers are separated by 2×10^6 bp, to walk from R_L to R_R would require at least 60 steps (allowing 20 per cent of cosmid overlap per step), which would be a formidable task. An exacerbating factor is that it is not normally possible to orientate R_L and R_R with respect to each other, and thus it is required to walk from R_R and R_L in both directions simultaneously, making the number of steps required twice as large — in the case of our procedure, a total of at least 120. The technique becomes much more attractive where several different 'starts' for the walk are available as well as R_L and R_R . Each walk can then be spread out from four or five starts, considerably reducing these difficulties.

Jumping has been suggested as a method that would reduce the number of steps that are required to walk large distances to an acceptable level — large jumps would be taken rather than individual steps¹¹. Consider a 200,000-bp linear DNA molecule (Fig. 2). If it were possible to mark one end of the molecule with a short DNA sequence (M) and then to join the whole molecule into a circle, pieces of DNA (A and B) that were originally 200,000-bp apart would be on either side of the marker DNA. If many such random 200,000-bp circles (representing the whole human genome) could be constructed, and if the marker DNA plus flanking sequences could then be isolated, a 'jumping' library representing all DNA sequences 200,000-bp apart would be produced. We could isolate R_L from the library and 'jump' down the chromosome until we found R_R .

Collins (personal communication) and Lehrach (personal communication) have very recently overcome the considerable

technical difficulty involved in constructing statistically complete jumping libraries. Their libraries will allow rigorous testing of the frequency of artefactual joining of sequences by inter- rather than intramolecular fragment joining and of the distance and difficulty of each jump. The use of these techniques, together with orthogonal- or inverting-field gel electrophoresis to locate jump sites, should enormously improve our chances of isolating the defective gene.

I have not approached the obvious question of how one can identify a single, defective gene in 2×10^6 bp of cloned DNA. The answer to this depends very much on the gene and the defect and will prove to be a technical challenge.

One question remains concerning all these approaches: are all human DNA sequences clonable in *Escherichia coli*? The answer is that most are but that some,

probably less than 5 per cent, may not be. Wyman *et al.*¹² show that about 5 per cent of human DNA fragments cloned into lambda phage vectors will not propagate on the normal lambda phage *E. coli* hosts but require specific *E. coli* mutant strains for successful growth. The problem will probably be overcome by careful strain construction and testing.

In conclusion, it is certain that RFLPs will lead to the affected gene. Which route is taken in each case depends on the disease pathology, the chromosome location and the tenacity of the investigator, but the search will eventually be successful. It is to be hoped that the good luck which has accompanied some attempts at RFLP mapping will extend to the search for affected genes. □

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Radiation detectors

Wirechambers in Vienna

from David J. Miller

BOTH 'wirechamber families' are growing; the family of users and the family of radiation detectors which they use. The Vienna meeting* serves as a reunion every three years for a worldwide community of astrophysicists, plasma physicists, medical physicists and biologists as well as the particle physicists who were the first to use proportional chambers and tubes.

The basic principle of all wirechamber devices is multiplication of the electrons released when a gas is ionized by radiation. In true wirechambers multiplication occurs in very high electric fields around fine-sense wires at positive high voltage. In parallel-plate chambers the multiplication is in more uniform fields between plates or grids. Some devices are best adapted to the detection of charged particles, but there are important developments in the detection of photons, especially at ultraviolet and X-ray energies.

Biologists are using multiwire chambers with X-rays from synchrotron sources (A.R. Farouki, MRC Laboratory of Molecular Biology, Cambridge) and with neutron beams (B.P. Schoenborn, Brookhaven National Laboratory). In both cases the chambers were developed by groups with their roots in high-energy physics. The Brookhaven chamber is filled with up to six atmospheres of ³He, one of the few substances which detects slow neutrons. The chamber has a spatial resolution of just over a millimetre, which is close to ideal for resolving the neutron-diffraction patterns from laminated structures from, for example, frog retinas or

crystals of complex proteins.

Recent developments in parallel-plate avalanche chambers for X-ray astronomy were discussed in a review by G. Charpak (CERN, Geneva), referring to work from the Mullard Space Science Laboratory of University College London and the University of Leicester. A minority gas (usually acetylene) has been used to enhance the avalanche gain and to reduce statistical fluctuations in measurement of X-ray energies from pulse height. Very simple readout with good position resolution has also been achieved by using fine intersecting patterns of wedges and strips on the anode. A. Breskin (Weizmann Institute, Israel) has achieved a gain of 10^6 in a multistep low-pressure parallel-plate chamber, and plans to use the technique to detect ultraviolet photons from cerenkov rings produced by electrons in a fixed-target experiment at CERN.

'The golden egg of Chicago', a large detector for primary cosmic rays which has been flown on the Space Shuttle (D. Mueller, Enrico Fermi Institute, Chicago), is a beautiful and complex device that contains cerenkov counters, scintillators and special transition-radiation detectors which are read out by xenon-filled multiwire proportional chambers. The detector already has results on the abundances of the lighter nuclei (such as C, N and O) in cosmic radiation, with a clear peak in the iron region, but there is much data still to be analysed. Wirechambers normally need a lot of tuning but these performed perfectly without any maintenance during the flight.

The new generation of storage-ring

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*The fourth Wirechamber Conference took place in Vienna on 25–28 February 1986.

experiments in particle physics has generated dozens of new devices with at least two radically different solutions on offer for every problem. Almost every experiment contains a large cylindrical chamber to measure the moments of charged particles, but half of them use time projection chambers (TPCs) and the other half jet chambers. A TPC is a completely clear gas volume with a multiwire detector at one end onto which electrons drift in a uniform electric field. It is, in principle, the ideal three-dimensional detector, but the first big TPC had many teething troubles and has only begun to work well in the past two years (D. Nygren, Berkeley, California). Meanwhile the jet chambers, which use shorter drifts and large numbers of wires stretched throughout their volume, have accumulated a very good record of reliability. Their advocates claim that these chambers can resolve bundles (or jets) of closely correlated tracks much better than TPCs. The conference heard detailed reports on new developments in TPCs from Japan and on two detectors for the large electron-positron (LEP) collider at CERN, Geneva. There are new jet chambers for the linear electron-positron collider (SLC) at Stanford, California, for the Cornell University e^+e^- collider (CESR) and for the other two LEP experiments. Each of these jet chambers has different wire geometry and different electronics. It will be fascinating to see who has guessed right.

Perhaps the most ambitious detectors of all are the ring-imaging cerenkov (RICH) counters for the DELPHI experiment at

LEP (J. Seguinot, College de France; M. Turala, University of California, Santa Cruz). As in Breskin's detector, the ultraviolet photons from high-energy tracks ionize an organic vapour (TMAE). Electrons from the ionization process then drift for very long distances before reaching the sense-wires. Apart from the mechanical problems of constructing large-area thin mirrors and windows for these counters, there is a fundamental problem in RICH counters arising from the multiplication process. Electron multiplication releases ultraviolet photons that can interact with the TMAE to produce extra spurious electrons. Test results suggest that this problem can be solved by shielding each sense wire from its neighbours with little 'fences' or tubes.

One of the most exciting potential applications of wirechambers is in positron emission tomography. Good spatial resolution is needed for the 511-KeV γ rays from electron-positron annihilation, with a fast data-collection rate to accumulate a picture of the distribution of the positron-emitting isotope inside an immobilized patient. Chrapak thinks it can be done with barium fluoride crystals and parallel-plate avalanche chambers. But there are problems as well as several other competing techniques such as the use of bismuth germanate crystals with solid-state photodetectors. There is a good chance that a successful system will be working before the next family reunion in 1989. □

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Immunology

A common form of killing

From Peter J. Lachmann

IT HAS been established for some years that serum complement kills cells by the insertion of pore-forming molecules (plugs) into the target membrane and the suggestion that lymphocytes kill target cells by an analogous process, discussed in a *News and Views* article¹ some time ago, has been amply confirmed^{2,3}. Certain bacterial toxins — for example streptolysin-O and staphylococcal alpha-toxins act in a similar way⁴ as does the bee venom toxin, melittin, whose pore-forming activity is rather similar to that of complement⁵. The paper by Young *et al.*⁶ on page 613 of this issue extends the role of pore-forming molecules to the eosinophil, a cytotoxic cell of the myeloid series which is known to have important cytotoxic activities both in defence against parasitic infections and in the pathogenesis of the cardiac lesions associated with eosinophilia.

The evidence presented by Young *et al.* is based largely upon electrical potential

changes in membranes and lipid monolayers, techniques which have been used by other groups to demonstrate pore formation by complement and by this group to confirm the existence of such molecules in the granules from cytotoxic T cells and natural killer cells^{7,8} as well as to demonstrate similar pore-forming molecules in *Entamoeba histolytica*¹⁰. Marker release from liposomes can be demonstrated and molecular sieving is now being used to find the size of the functional lesion. The ultrastructural appearance of the lesions produced by the membrane attack complex of complement, the final pore-forming components, and by perforins, the leukocyte equivalents, has also been extremely helpful in elucidating their nature and it will be of interest to see what the eosinophil pore looks like when seen under the electron microscope.

That the eosinophil can form pores is of particular interest because, like other

granulocytes, the eosinophil has another major killing mechanism available to it — the production of oxygen metabolites producing what may be termed 'burns'. Oxygen metabolites formed by the 'superoxide burst' are known to be the principal intraphagosomal killing mechanism for microorganisms¹¹. Oxygen metabolites are also implicated in extracellular cytotoxicity brought about by neutrophils and by macrophages¹². These reactions produce their membrane damage finally by lipid peroxidation and can characteristically be inhibited by catalase or superoxide dismutase or by iron chelators. Because the eosinophil contains a peroxidase in its granules and is capable of producing a superoxide burst, it is likely that it also uses oxygen metabolites for cytotoxicity in some circumstances. It is therefore a clear example of a cell that has, and seems to use, two of the major cytotoxic mechanisms.

The third major cytotoxic mechanism of eukaryotic cells involves the production of soluble cytotoxic factors, of which tumour necrosis factor and lymphotoxin are the best described. It is clear that monocytes use tumour necrosis factor when they kill nucleated cells by antibody-dependent cellular cytotoxicity¹³⁻¹⁵ and although the roles of lymphotoxins in killing by T cells (or B cells) remain to be clearly defined, these cells can certainly produce lymphotoxin, which presumably does function *in vivo*.

It is therefore now becoming clear that these three major mechanisms — plugs, burns and poisons — are used in various combinations by different cell types and in different circumstances. Young *et al.* show that eosinophils can use plugs as well as burns. Lymphocytes use plugs as well as poisons and macrophages certainly use burns as well as poisons. It will be interesting to see if any cell uses all three mechanisms. □

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Physics

Low-dimensional electron movement in a magnetic field

from Paul Chaikin

ELECTRONS in two dimensions in a perpendicular magnetic field move in circular orbits. Quantization of these orbits is responsible for the quantum Hall effect as well as a wealth of oscillatory phenomena in metals. But electrons in one dimension in a magnetic field cannot form such orbits. The discovery of a series of magnetic field-induced phase transitions, oscillations and behaviour like the quantum Hall effect in Bechgaard salts, which act as quasi-one-dimensional organic conductors, has stimulated a flurry of recent experimental¹⁻⁵ and theoretical⁶⁻⁹ work and the discovery of a new form of instability in a two-dimensional system^{10,11}, as well as many unanswered questions.

The crystal potential has two interesting effects on the states of a two-dimensional system in the field. If the potential is reasonably isotropic (as in a square lattice) the electron orbits are distorted but closed and periodic. The wavefunctions must satisfy both orbital quantization and crystalline symmetry, conditions which are usually incommensurate. The result is a complex hierarchical self-similar energy spectrum with gaps on all energy scales^{12,13} (Fig. 1). If the potential is highly anisotropic the motion of the electron may form a zigzag pattern which goes off in the easiest direction and never closes — an open orbit (Fig. 2).

The Bechgaard salts provide a unique system for the study of an interacting two-dimensional electron gas in a magnetic field. They have the formula (TMTSF)₂X where X is PF₆, ClO₄, ReO₄ and so on¹⁻⁵, and are variously regarded as quasi-one- or quasi-two-dimensional. They are better known as the first organic superconductors, but they can also have insulating spin-density wave (SDW) ground states depending on the pressure and on the anion X. The band structure has been calculated and measured: the anisotropy in bandwidths in the highly conducting plane

is about 10:1, whereas between the planes the bandwidth is smaller by a factor of 30.

It is well known that one-dimensional metals are unstable against a Peierl's distortion or SDW which leads to an insulating ground state. For the Bechgaard salts under consideration the band-

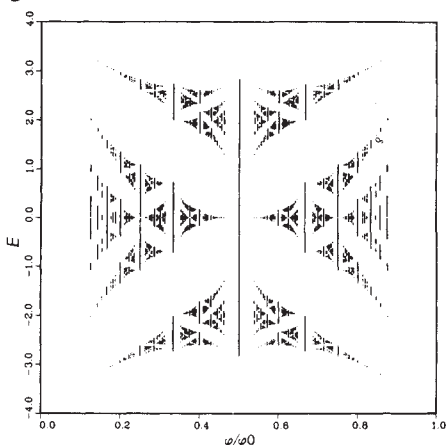


Fig. 1 Energy spectrum of an electron in a square tight binding model in the presence of a magnetic field measured in flux quanta per unit cell (ϕ/ϕ_0). Dark regions, allowed states.

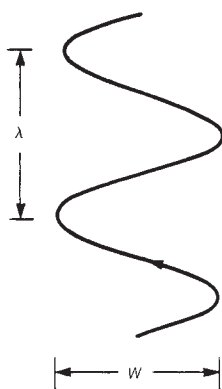


Fig. 2 Electron motion in a magnetic field for an open orbit. w and λ are proportional to the reciprocal of the magnetic field.

width in the second most conducting direction is large, the systems are effectively two-dimensional and they remain metallic to low temperatures. But the anisotropy is sufficient to make all the orbits open at the Fermi surface. In the presence of a magnetic field the electrons undergo the motion illustrated in Fig. 2. As the magnetic field increases the width w of the motion along the x direction and the period λ along the y direction decrease. The effect is to make the electrons more one-dimensional and thus increasingly susceptible to an SDW transition. Strictly speaking, the electron motion and

the spectrum of the open-orbit electrons in a magnetic field is always one-dimensional (limited excursions along x and infinite along y). Thus, two-dimensional open-orbit metals cannot exist at zero temperature. An infinitesimal magnetic field will drive them to an insulating Peierl's or SDW state^{12,13}.

The basic instability leading to the field-induced transition is therefore well established. There are several quasi-classical models that seek to explain the series of phase transitions which allow the wavevector of the SDW distortion to change both continuously and discontinuously to take advantage of the magnetic quantization of carrier pockets which result from the distortion⁶⁻⁹.

In the usual treatment of the one-dimensional instability a single length is considered — the reciprocal of the Fermi momentum (P_F) — and this sets the wavelength of the distortion at $\hbar/2 P_F$. In the field-induced phase there at least three lengths, $\hbar/2 P_F$, the magnetic length λ (Fig. 2), which varies reciprocally with the magnetic field, and the lattice period. In general the lengths are incommensurate with one another. The distortion wavevector results from the competition of the interactions represented by these lengths. The spectrum resulting from incommensurate potentials is often quite complex, as illustrated for a particular case in Fig. 1.

One might expect that the existence of the small gaps in the spectrum are inconsequential, as they are usually smeared out by finite temperature or scattering. The gaps have a profound effect on the Hall conductance, with the most dramatic effects involving large changes in magnitude and sign of the Hall conductance resulting from the smallest gaps¹⁴. More perfect crystals show an increasing number of field-induced transitions with abrupt sign changes in the Hall voltage¹⁵, suggesting that in these salts an incommensurate spectrum is involved³. The existence of oscillations with a different magnetic frequency at high fields also points to a complex spectrum².

There are still basic questions left to be answered. The eventual state at high field should be insulating, but so far only semi-metallic behaviour has been observed. And what of the quantum Hall effect? Hall measurements indicate steplike structure, but it is temperature dependent and has other dissimilarities¹⁵⁻¹⁷. The interplane bandwidth is small enough to allow the two-dimensional instability to occur, but is the system two-dimensional enough for the quantum Hall effect? The roles of incommensurability and dimensionality need further investigation. □

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Desert ecosystems

Desertification or xerification?

from N. E. West

'DESERTIFICATION' has become a common term to describe the impoverishment of terrestrial ecosystems induced by man. But the word is much abused, often preventing the realization that deserts occur naturally in certain ecological contexts. Although the impression that deserts are produced by man helps to generate interest, and therefore money, to investigate problems of vegetation diminishment and soil degradation, particularly in regions of semi-arid climates^{1,2}, it encourages emphasis on treating the symptoms of abuse rather than the causes of the problems.

Deserts vary enormously around the world, with only some of those under the most arid conditions having escaped severe alteration by man^{3,4}. Le Houterou⁵ proposed the term 'desertization' to imply the expansion of desert-like landforms and landscapes in arid to semi-arid environments. This word is supposed to avoid issues such as ecological degradation of tropical forests into savanna of forest into maquis, which can still be described as 'desertification'. But the distinction is probably missed by most and, in any case, degradation of terrestrial ecosystems usually does involve drying-out of the previous environment. Water typically enters the biological phases less easily, yet moves through an ecosystem

more rapidly, following disturbance introduced by man. If soils are more compacted and organic matter lower, less water can infiltrate and be stored. Accelerated erosion of the soil surface may reduce the depth of the rooting profile as well as removing the most nutrient-rich portions. More xerophytic (drought-tolerant and less water-demanding) plants typically replace the preceding comparatively less xerophytic ones removed by ex-

cessive livestock grazing, wood harvest, fire and mechanical damage. This gives the impression that macroclimates must have changed when the major alterations in climate have occurred at soil level. Kovda⁶, therefore, proposed the term 'aridization' to describe the drying-out process. The disadvantage of this word is that it can be confused with a permanent change in macroclimate.

I have recently used the term 'xerification' to describe the concomitant diminishment of vegetation and degradation of soils under unsustainable levels of land use in semi-arid areas in western North America⁷. This term could be used in more climatically favourable regions without the value-judgement implicit in

Albert L. Lehninger (1917–1986)

ALBERT LEHNINGER, who died on 4 March, is known to young and not so young biochemists all over the world as the sole author of the widely used textbook, simply called *Biochemistry*, the first edition of which appeared in 1970. To the older biochemist and to all those engaged in the study of the branch of biochemistry and biophysics called bioenergetics, he is known as one of the founders of their field. He was one of the first to publicize the word bioenergetics in his monograph bearing that name, published in 1965. His first book, *The Mitochondrion*, had appeared a year earlier.

It was his work on this sub-cellular organelle in the late 1940s and the 1950s that first brought him fame. Together with E.P. Kennedy, he showed in 1948 that a particulate fraction of rat liver, identified as mitochondria, catalyses all the reactions of the Krebs cycle and fatty acid oxidation. For this work Kennedy and Lehninger are co-recipients of the 1986 Passano Foundation Award.

Lehninger is perhaps best known for his second classical paper, also published in 1948. In that paper, together with his student Morris Friedkin, he described phosphorylation of adenosine diphosphate by inorganic phosphate, coupled to electron transfer between reduced nicotinamide nucleotide and oxygen (catalysed by the so-called respiratory chain). The phenomenon of oxidate phosphorylation itself had already been established by Engelhardt and Kalckar in the 1930s and before the Second World War Ochoa and Belitzer had made it likely that it was of a fundamentally different type from the only known phosphorylation reaction, linked with anaerobic processes. But the direct demonstration of what became known as respiratory-chain phosphorylation had proved elusive. Lehninger succeeded in two types of experiments, one with reduced nicotinamide-adenine dinucleotide as substrate and the other with 3-hydroxybutyrate, which reduces the

nucleotide in a reaction that could not conceivably be associated with phosphorylation.

With the hindsight of nearly 40 years, we now know that the results of the first type of experiment were not as unambiguous as they at first appeared, but the second type remain valid and were sufficient to establish the concept of respiratory-chain phosphorylation. Lehninger was also the first to demonstrate phosphorylation in the terminal reaction of the respiratory chain — the oxidation of reduced cytochrome *c* — once again after failures by others. He was also one of the first to observe the energy-linked uptake of calcium by mitochondria and the first to make a thorough study of its characteristics.

Lehninger was one of a vanishing type: the highly successful research worker who is also a prolific writer. Also unusual is that all his books are monographs. How he was able to face up to the tremendous chore of regularly bringing out new editions of his monumental textbook was a source of wonder to many. Surely the explanation is that he enjoyed writing. That he was a such a lucid writer was no surprise to those of us who used to listen spellbound to his lectures at international meetings, delivered without any notes. The polish of these performances could only have been achieved with meticulous preparation.

Lehninger's early work was done at the University of Chicago where he moved in 1945 after obtaining his Ph.D. at that nursery of American biochemists at that time, the University of Wisconsin. He spent the academic year 1951–1952 at the University of Cambridge, England, and in 1952 was appointed Professor of Physiological Chemistry at Johns Hopkins University, Baltimore, where he stayed until his death. His colleagues at Johns Hopkins had planned a symposium, appropriately called *The Mitochondrion*, 1986, to be held in his honour on 5–7 June. The symposium will still be held, now sadly to his memory as well as in his honour.

E. C. Slater



LANDSAT imagery of the boundary between Egypt and Israel as it appeared in 1973. 1, sand, vegetation destroyed; 2, sand occupied by intact vegetation; 3, Mediterranean Sea; 4, shifting sands next to coastal strands; 5, sandy area covered by orchards in southern part of Gaza; 6, fenced area of Sadat and Vetiv ha Asara where grazing and direct plant cutting ceased after 1967. From Danin, A. *Desert Vegetation of Israel and Sinai* (Cana, Jerusalem, 1983). Image provided by NASA.

words such as desertification, desertization and aridization.

Irrigated lands in arid to semi-arid climates which have increased water tables and/or direct salt input from the irrigation water eventually support lowered crop production. Even some originally forested lands in southern Australia develop such problems when the high transpiration draw-downs affected by the forest are eliminated after tree removal. The only plants that can grow on some of the most heavily salinized former farm- or forestlands are shrubs normally found in desert regions. The soil moisture is excessive, but the soils are physiologically dry to non-desert species. The term xerification would more usefully describe these processes than the narrow word salinization.

Clearly it will be difficult to distinguish natural xerification from that induced by man. But palaeoecological evidence shows that deserts are relatively young ecosystems on all continents, and thus have arisen naturally. Therefore it is important to elucidate natural, as well as man-made, mechanisms. □

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Muscle contraction

Crossbridges, force and motion

from Richard T. Tregear

THE basic question that concerns those interested in crossbridges is 'what makes muscle pull?'. Many different answers have been suggested over the years. Some simple and elegant experiments described at a recent workshop* exclude several of them. Other experiments place constraints on the part of the myosin head (or crossbridge) responsible for the generation of force consequent on ATP hydrolysis. The picture which emerges is of a crossbridge (which projects from the thick filament of muscle fibres) that can easily attach and detach from actin (in the thin filament) in the first part of the enzymatic cycle and then 'harden' its attachment and produce force in the later part of the cycle. The contractile process occurs in the middle of the myosin head, does not need the other head of the myosin molecule, much of the tail region of the molecule or even, perhaps, the light chains of the head itself. The process probably involves only a small change in the shape of the head, is reversible and can sometimes couple to a huge interfilament movement. Altogether this picture is not very like the usual undergraduate idea of crossbridge rotation and force production.

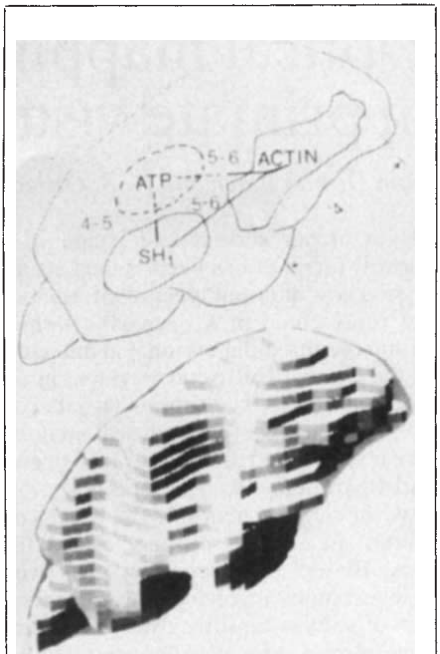
The evidence for these statements comes from a wide variety of approaches. The idea of the rapidly attaching weak-binding crossbridge comes from enzymatic studies (E. Eisenberg, NIH), and fits both with the way in which measurements of crossbridge attachment by X-ray diffraction (H. Huxley, Cambridge; K. Wakabayashi, Osaka) and mechanical stiffness (R. Simmons, London) are seen

to rise before tension during activation and with the large number of disorganized myosin heads still seen by electron proton resonance (EPR) when a muscle is contracting strongly (D. Thomas, Minneapolis). On the other hand, the strong-binding, tension-generating state may be best seen by electron microscopy of muscle in rigor (M. Reedy, Duke); at any rate, no regular crossbridge angle other than that of rigor (thought to represent the maximal force-producing state) has been detected by EPR or polarized fluorescence in actively contracting muscle.

The resolution of immuno-electron microscopy is now good enough to divide the myosin head into a regulatory neck and an operative body, and within that body to locate the three regions most likely to transfer energy across it: the nucleotide- and actin-binding regions and the interlinking part close to the active thiol residue (T. Wakabayashi, Tokyo; see figure). Furthermore, both the sequence near the thiol and the purine-binding sequence have turned out to be highly conserved, being invariant from slime mould to rabbit, as if they perform an essential function (J. Spudich, Stanford).

Optical microscopy has now improved to such an extent that one can see the motion of unrestrained actin filaments over myosin, or of particles coated with myosin over actin (Spudich; T. Yanagida, Osaka). Such motion occurs even when the myosin is single-headed or its tail is greatly reduced in length. These experiments appear to eliminate contractile mechanisms dependent on either head-head interaction¹ or a change in tail structure².

Chemists have managed to attach a photolysable group to ATP (caged ATP) that can be diffused into muscle before



Location of the ATP-binding and reactive thiol (SH) sites on the surface of the myosin head (top), determined from avidin binding and three-dimensional reconstruction of the actin-myosin head complex (bottom). ATP binds to the back of the head. Units, nm. (Courtesy of M. Tokunaga, A. Tomioka, C. Toyoshima, K. Sutou, K. Yamamoto & T. Wakabayashi, unpublished observations.)

lysis releases the ATP itself³. The resultant synchronized events indicate that phosphate release and tension generation occur together and that both processes are reversible (Y. Goldman, Philadelphia). It follows that tension can be regenerated without coupled ATP hydrolysis, which was not envisaged in earlier ideas of the crossbridge cycle⁴.

An ingenious argument was presented from a couple of conceptually simple experiments. If actin is proteolytically cut loose in a myofibril or if myosin heads are floated into a brush of actin filaments, they remain still until ATP is added and then they move, quite rapidly. Yanagida measured the speed of motion and the rate of ATP hydrolysis and deduced that each crossbridge stroke using ATP moves the myosin molecule by at least 60 nm, which is too far for the crossbridge to stretch^{5,6}. Is there something wrong with Yanagida's logic, or with the crossbridge theory itself? □

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* Workshop on the interaction between actin and myosin in skeletal muscle held at Alpbach, Austria, 17-22 March 1986. Supported by the European Molecular Biology Organization and the Muscular Dystrophy Association of America.

Neuroscience

Optical mapping of activity in primate visual cortex

from D. Van Essen and H. S. Orbach

MUCH of our current understanding of cortical function has been gained by analysing how different types of information are represented in a systematic fashion within the three-dimensional architecture of the cortex. This is most evident in the case of primary visual cortex (striate cortex) of primates, where cortical architecture is specialized to a notably high degree and where many of these specializations have interesting neurophysiological correlates. In a report on page 579 of this issue, Blasdel and Salama¹ use a relatively new technique involving *in vivo* application of voltage-sensitive dyes to ascertain how selectivity for stimulus orientation is mapped across the surface of striate cortex in the macaque monkey. Their study provides an intriguing and unexpected hint about modular organization of the cortex. In addition, it emphasizes the feasibility of the optical approach for attacking a host of interesting issues concerning cortical function.

It is well known² that cells in the striate cortex are arranged in orientation columns, in which aggregates of cells aligned orthogonal to the cortical surface all respond best to the same stimulus orientation (except for cells completely lacking orientation selectivity). Parallel to the cortical surface, orientation preferences tend to shift gradually and smoothly, with only occasional discontinuities in the progression. A major unresolved issue has been the relationship of this orderly array of orientation columns to two other basic features of striate cortex: a system of ocular dominance stripes that is most clearly delineated by a sharp segregation of geniculocortical inputs from the two eyes within layer 4; and a patchwork array of cytochrome oxidase-rich 'blobs', which are aligned with ocular dominance stripes and whose constituent cells generally lack orientation selectivity³.

What has clearly been needed to sort out these relationships is a means for monitoring the two-dimensional pattern of visually evoked cortical activity with high spatial resolution. The 2-deoxyglucose technique, in which activity-dependent metabolism is measured autoradiographically, has proven helpful in this respect⁴. But it suffers from being a one-shot approach, in which only a single stimulation condition can be tested in any given cortical region. The technique of *in vivo* optical monitoring of neural activity using voltage-sensitive dyes is in principle much more flexible, because it has vastly super-

ior time resolution and allows repeated measurements using various stimuli. First developed and tested on invertebrate preparations by Cohen and collaborators⁵, voltage-sensitive dyes have recently been successfully applied to the mammalian somatosensory and visual cortex^{6,7}.

Several methodological refinements introduced in the study of Blasdel and Salama¹ yield a spectacular increase in the spatial resolution of the technique. Most importantly, the authors used a video camera instead of the standard photodetector array, and monitored a very slow visually evoked response, thereby gaining spatial resolution in exchange for an affordable loss of temporal resolution. In addition, they used a clever combination of stimulation paradigms and image-processing techniques to generate detailed false-colour maps of how orientation preferences are mapped across the surface of the cortex. The basic validity of this analysis was confirmed by direct comparisons between optical and electrophysiological determinations of ocular dominance and preferred orientation at selected sites.

Blasdel and Salama find that regions of similar orientation preference generally occupy small patches in the orientation map. These patches range from nearly circular to highly elongated in shape, with a length typically less than 1 mm. The alignment of iso-orientation domains is not strongly correlated with ocular dominance stripes nor with cytochrome oxidase blobs, an outcome which is consistent with previous deoxyglucose-based results⁴. It runs counter to more organized patterns that have been hypothesized, such as an orthogonality between ocular dominance and orientation stripes⁷ or a radial or circumferential arrangement of iso-orientation domains centred around cytochrome oxidase blobs⁸.

A particularly striking feature of the orientation maps is a pattern of 'fractures', lines or narrow strips along which the preferred orientation changes very rapidly. The fracture lines form a tightly interlaced network containing numerous loops and rings that are variable in size and shape, but are generally much less than 1 mm across. Blasdel and Salama raise the intriguing suggestion that these fracture lines form the outlines of discrete 'modules' within the cortex. However, they also note several potential complications relating to the fracture lines, and these deserve careful evaluation.

One of the complications arises from the fact that the maps display a well-defined orientation at all points, even in regions which post-mortem histochemistry shows to be superimposed on the cytochrome oxidase blobs. This is surprising in view of the previously mentioned complete absence of electrophysiologically assessed orientation selectivity in most blobs³. One possibility is that orientation selectivity might indeed be present in the blobs, but restricted to the most superficial cells (say, in layer 2), which presumably dominate the optical signals. Alternatively, the apparent orientation selectivity within the blobs might arise from spatial blurring of the optical signal, for example, as a result of light scattering within the cortex or as a reflection of the extent of dendritic arborizations. By this hypothesis patches whose cells genuinely lack orientation selectivity would nonetheless show an apparent orientation preference throughout, by virtue of spatial averaging of the orientations of nearby sites within the blur circle. Fracture lines associated with blobs would then no longer represent an abrupt discontinuity in a parameter that has a well-defined value at each point, but rather a mismatch in the extrapolated orientation in going from one side of a blob to the other.

Another puzzling feature is that not all orientations are equally represented within either of the two orientation maps illustrated by Blasdel and Salama. In particular, one oblique orientation (light blue in their false-colour representations) appears to be notably sparse, generally occurring as very narrow strips. Non-linearities in the colour reproduction process may contribute to this impression but are unlikely to provide a complete explanation, because many of the fracture lines run directly along the thin strips associated with the under-represented orientation. There may be pronounced anisotropies in the cortical representation of orientation, analogous to those suggested from electrophysiological recordings⁹. Yet another possibility is that the apparent under-representation was a consequence of slightly reduced efficacy of a particular stimulus orientation, conceivably because of astigmatism of one or both eyes. A final complication to the fracture story is that some of the fracture lines (more than seems likely by chance association) appear by visual inspection to parallel closely the vascular pattern (including some of the smaller blood vessels) that is shown in a figure accompanying the study. This may reflect yet another way in which non-neural factors can subtly bias the orientation estimates derived from optical measurements.

The question of whether striate cortex contains well-defined modules is fundamental to our understanding of cortical organization. Hubel and Wiesel^{2,4} laid the

groundwork for this notion with the suggestion that any small region of cortex, about 1–2 mm on a side, contains a set of 'hypercolumns', that is, all the neural machinery needed for the analysis of orientation and ocular dominance in any small portion of the visual field. The idea received a major boost with the discovery of the cytochrome oxidase blobs, which clearly denote an anatomically distinctive two-dimensional repetitive pattern, but which leave open the question of whether hypercolumns have well-defined starting and stopping points.

The orientation fracture lines described by Blasdel and Salama are attractive candidates for hypercolumn boundaries. But the relationship is not entirely straightforward, as most of the modules delineated by fractures fail to enclose a complete set of orientations, and do not all contain a representation of both ocular dominance stripes. A more precise understanding of this relationship must await resolution of the technically based concerns described above about the origins and interpretations of some of the orientation fracture lines.

These concerns are amenable to experimental analysis, and clarification of the key issues should not be long in coming. Further refinements may also be anticipated for dealing with various technical limitations of the optical technique relat-

ing to signal-to-noise problems, image blur and the analysis of signal distribution within the third dimension (depth) of the target tissue.

In the meantime there is good reason to celebrate the successful application of voltage-sensitive dyes to basic questions of cortical organization. Blasdel and Salama have made clear that optically based activity monitoring techniques will become a powerful component in the ever-expanding repertoire available to neuroscientists interested in structure–function relationships within the brain. Application of this approach to questions of information processing, development and plasticity in sensory and motor areas of the cortex will keep many laboratories busy for years to come. □

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Molecular biology

Why do disused proteins become genetically lost or repressed?

from Jared M. Diamond

ARTICLES in these columns typically focus on recent discoveries. But progress also depends on exposing areas of ignorance. Hence this article concerns our failure so far to resolve a fundamental problem of biology: why disused proteins become phenotypically repressed and are eventually genetically eliminated^{1–5}.

Evolution consists of the acquisition and loss of traits. Most biologists since Darwin have agreed that the acquisition of functionally significant traits is caused by natural selection. But why do disused, formerly significant traits become lost? (Think of the loss of eyes in cave animals, or the loss of flight in birds on predator-free islands.) Two answers have been suggested, both stemming from Darwin's work: disuse or natural selection. Most mutations destroy a function rather than improve it. Thus, if a character becomes selectively neutral through disuse, mutations will gradually remove the character even in the absence of positive selection to eliminate it. On the other hand, any character costs something to build and main-

tain. Thus, a disused character may be selectively disadvantageous, not merely neutral; selection may eliminate the character to recoup the wasted costs. The cost most often mentioned in this context is biosynthetic energy.

The traits whose costs could be measured most easily are single enzymes or biosynthetic pathways. Consider auxotrophic mutants, which have lost the ability to synthesize some essential solute and can grow only if that solute is provided exogenously. Lwoff⁶ proposed that there should be positive selection for such mutants in the presence of the solute because the mutants save unnecessary biosynthetic costs incurred by the wild strain. Zamenhof and Eichhorn⁷ confirmed that His[–] and Trp[–] auxotrophs of *Bacillus subtilis* are selected over the wild-type strain in the presence of His and Trp, respectively. Similar findings apply to Tyr[–] and Trp[–] auxotrophs of *Escherichia coli*^{1,8}. Few attempts have been made to compare the saved biosynthetic costs of the auxotroph at the molecular level with

its selective advantage at the population level, and thereby to decide whether the wasted energy hypothesis suffices to explain this genetic loss of a disused trait. The rare attempts that have been made cast doubt on the energy hypothesis^{1,2}.

The most detailed comparison is one in which Dykhuizen¹ used a chemostat to study *E. coli* mutants differing from the wild type only in their inability to synthesize tryptophan (Trp). Dykhuizen first calculated the energy needed by wild-type *E. coli* to synthesize various amino acids, assuming maintenance costs to be negligible compared with the costs of growth⁹. Amino-acid synthesis was estimated to cost 40 per cent of the total energy budget, Trp, in particular, 1.25 per cent. In a Trp-containing medium, the synthesis of this amino acid is repressed to a level where it costs only 0.01 per cent of the energy budget. The energy to synthesize the Trp-synthesizing enzymes themselves (in the absence of turnover) was estimated as only 17 per cent of this cost of Trp synthesis. Thus, if the sole advantage of the auxotrophs over the wild type in a Trp-containing medium were the saved synthetic energy, the auxotrophs should have had an insignificantly higher growth rate, by 0.01 per cent per generation (independent of generation time). In fact, their growth rate advantage was far greater — around 10 per cent per generation — and it increased with generation time.

Because these calculations of saved energy involve many assumptions, Dykhuizen next compared the selective advantage (over the wild type) of a mis-sense mutant that makes Trp-synthesizing enzymes but not the protein against a polar nonsense mutant that makes neither the enzymes nor Trp. By the saved energy hypothesis the polar nonsense mutant should have had a 17 per cent greater selective advantage than the mis-sense mutant (because energy savings are 17 per cent greater), but the two mutants were found to have the same advantage. Furthermore, mutants can still synthesize Trp from anthranilate via indole, although at some biosynthetic cost. Hence the selective advantage of the mutants over the wild type should decrease from a Trp to an indole to an anthranilate medium, and Dykhuizen, from the biosynthetic costs of each step, predicted relative advantages of 1.00:0.83:0.67. The actual relative advantages were the same for indole and anthranilate, and were lower than predicted (1.00:0.50:0.50).

Most puzzling of all, the advantage of mutants over the wild type was apparent only when they were grown together; the mutants and wild type had the same growth rates when grown separately. One can think of many uncertainties in these cost estimates: assumptions in the energy calculations that would affect the numbers; features of cell physiology that could

amplify a biosynthetic energy difference; and factors other than biosynthetic energy that should also be considered costs of Trp synthesis (for example, the space that Trp synthesis preempts in the already crowded cytoplasm or membrane^{3,10}). But all these considerations still predict incorrectly that the mutants should grow faster than the wild type when grown separately. Thus, the advantage of mutants must instead involve some interaction with the wild type when grown together, such as competition for glucose, the limiting substrate.

Similar doubts concerning the energy hypothesis emerge from experiments on *lac* operon proteins^{3,11,12}. For example, Andrews and Hegeman¹² compared five strains of *E. coli* differing in production of *lac* operon proteins (from 0.6 to 3.6 per cent of total cellular protein) growing in media with a substrate other than lactose. The authors observed no significant correlation between selective advantage and percentage of wasted protein.

The traditional explanation in terms of saved biosynthetic energy appears to fail.

The familiar observation that synthesis of specific proteins becomes phenotypically repressed in the absence of requirement for the protein is also usually attributed to saved energy. Experiments are needed in which the selective disadvantage of a mutant that cannot repress an enzyme in the absence of its substrate is measured and compared with the saved energy or other postulated costs. □

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Earth sciences

Magnetotelluric evidence for subduction of seafloor sediments

from D. Ian Gough

NEW results from magnetotelluric soundings across Vancouver Island reported by R.D. Kurtz, J.M. DeLaurier and J.C. Gupta elsewhere in this issue (*Nature* **321**, 596; 1986) provide the first correlation of high electrical conductivity with a seismic reflector at the top of a subducted plate. The good conductor, at depths of 20–50 km down the sloping plate, is best understood as composed of seafloor sediments and volcanic rocks that contain brine in their pore spaces, and carried on the Juan de Fuca plate as it subducted beneath Vancouver Island. If saline water is thus subducted at many convergent plate boundaries, the implications for the continental crust and the tectonic processes in it are important.

The magnetotelluric profile reported by Kurtz *et al.* forms part of the first of a series of transects across major structures in LITHOPROBE, the Canadian national programme of deep crustal studies. The central discipline of the programme is seismology, supported by other geophysical and geological techniques. In southern Vancouver Island three seismic reflection sections of outstanding clarity have been secured, showing both the subducting plate and much detail in the layered rocks above it (Green, A.G. *et al. Nature* **319**, 210; 1986). The magnetotelluric stations in the study of Kurtz *et al.* were sited along two of the three seismic profiles. At three

stations, the top of the good conductor is correlated with a seismic reflector within 10 per cent of depths in the range 23–28 km. The results from all 18 stations in the study can be fitted to a two-dimensional model in which a sloping conductive layer correlates well with a seismic reflector interpreted as the top of the downgoing plate throughout the depth range 23–34 km. Such correlations facilitate recognition and interpretation of structures located in different physical parameters (such as elastic moduli and electrical conductivity). It is planned to obtain magnetotelluric profiles at high station density along future seismic reflection sections of LITHOPROBE on land.

Fluids in the crust are attracting increasing attention. Crustal water is of critical importance in the response of rocks to stress, in particular in the thrusting of rock sheets a few kilometres thick over areas of several thousands of square kilometres in continental collision zones such as the Himalayan, Alpine and Appalachian mountain systems. Crustal water has great economic importance in relation to metallic ore genesis and to maturation and migration of hydrocarbons. Such water is generally a brine (Fyfe, W.S. *et al. Fluids in the Earth's Crust* Elsevier, Amsterdam, 1978); the effects of the chemical reactions of this water with silicate minerals in the lower crust are being explored. A brine is

a very good electrolytic conductor, and provided that it fills interconnected pore spaces or fractures it will give high bulk conductivity in the rock (Shankland, T.J. & Ander, M.E. *J. geophys. Res.* **88**, 9475; 1983). This is the interpretation of the conductive layer beneath Vancouver Island offered by Kurtz *et al.*

Saline water probably exists in crustal rocks of all ages. In tectonically active regions close to ocean ridges and subductions the silicate mineral of lowest melting point may provide another fluid that fills cavities between the other silicate crystals, both in the upper mantle and locally in the crust. Such partial melt is generally a good electrolytic conductor, and will produce high bulk conductivity if it is in interconnected spaces (Shankland, T.J. & Waff, H.S. *J. geophys. Res.* **82**, 5409; 1977).

In the interior of British Columbia, 200–600 km north-east of Vancouver Island, a large regional conductive layer is known to exist in the lower crust and uppermost mantle. I have suggested (*J. geophys. Res.* **91**, 1909; 1986) that this Canadian cordilleran regional conductor is caused by partial melt at upper mantle depths and saline water in the crust above. The evidence for subduction of seafloor rocks containing brine presented by Kurtz *et al.* gives one reasonable explanation of the accumulation of brine in the crust of the hinterland of the subduction. If similar evidence is found in other zones of subduction, the new result may lead to better understanding of the mechanisms responsible for the transfer of water into other regions of the continental crust.

It is to be hoped that magnetotelluric soundings will be added to the collection of seismic reflection profiles in other regions so that structures can be sensed in terms both of electrical conductivity and of seismic-wave parameters. Either saline water or partial melt produces contrasts of one to two orders of magnitude in electrical conductivity, whereas the corresponding effects on seismic-wave velocities and attenuations are of the order of 10 per cent. Magnetotelluric soundings should therefore detect fluids of both kinds with much greater sensitivity than can be achieved by seismology. On the other hand, seismology gives better location of velocity interfaces and faults. The two methods are complementary, and much more powerful together than either alone, as demonstrated in recent studies of the crust beneath the Rhenish Massif in Germany (Meissner, R. & Wever, T. *Am. Geophys. Union Geodynamics Ser.* **13**, 31; 1986, and Jödicke, H. *et al. in Plateau Uplift* ed. Fuchs, K. Springer, Berlin, 1983), as well as in the Vancouver Island studies of Kurtz *et al.* and of Green *et al.* □

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Weather forecasts still tricky

SIR—The recent paper on 'Fractal characterization of inhomogeneous geophysical measuring networks' by Lovejoy *et al.*¹ and a discussion in *News and Views*² address the important problem of detectability of sparse atmospheric phenomena and, therefore, of the very feasibility of useful weather forecasting. Now, whatever the difficulty of its detection might be, a sparse phenomenon will exert an influence on the *time course* of a multitude of more systematic phenomena occurring over a wide range of space scales. The main reason for this is the fundamental instability of the atmospheric system³: a perturbation arising from an uncontrollable localized event and acting on a larger scale phenomenon pertaining to atmospheric circulation may give rise to an (exponentially) divergent history and to an altogether different regime, or to an aperiodic sequence of regimes perceived by an unaware observer as 'noise'.

Let γ be the precision with which the initial state of the system can be determined in phase space. Assuming that the atmosphere behaves as an unstable dynamical system we obtain the following estimate for the error growth after time t :

$$\gamma(t) = \gamma e^{Kt} \quad (1)$$

where K is the largest positive Lyapounov exponent (or more generally the Kolmogorov entropy).

Since our variables are distributed in physical space, an inadequate spatial resolution l will clearly induce an error in the estimate of the variables. Conversely, if for some reason their values are subject to uncertainty, it will be difficult to locate with precision the region of space from which these variables had initially emanated. We may therefore substitute, in our crude estimate, the phase space uncertainty γ by the precision l with which the initial state of the atmosphere can be determined in the physical space and write, instead of (1)

$$l(t) = l e^{Kt} \quad (2)$$

When the size $l(t)$ will reach a value of the order of the dimensions of the entire system, L , predictions concerning individual histories will become meaningless and should be replaced by statistical considerations. The time τ_m for which this will happen is⁴:

$$\tau_m \approx \frac{1}{K} \ln \frac{L}{l} \quad (3)$$

This gives a quantitative measure of the limits of predictability of the system. A significant feature of τ_m is its logarithmic dependence on the parameter l , and thus its relative insensitivity to space resolution. Going from a quasi-uniform to a fractal distribution of l or vice versa is not going to change this picture drastically.

So although detection of sparse phenomena is important in its own right, it is

worth stressing that even with a considerable improvement of the world detection network the problem of forecasting of the global state of the atmosphere is likely to remain as acute as ever.

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Activation of cellular calcium entry

SIR—A recent *News and Views* piece by Baker¹ discusses the mechanisms of intracellular calcium ion (Ca^{2+}) release from the endoplasmic reticulum (ER) but refers briefly to cellular calcium entry from the extracellular fluid as being mediated by Ca^{2+} channels gated either chemically or by voltage. Voltage-activated Ca^{2+} channels that can be modulated by intracellular messengers have been characterized in many systems², but are unlikely to be present in a number of different epithelial tissues such as exocrine glands and liver simply because they are unable to fire action potentials and many activating hormones or neurotransmitters evoke membrane hyperpolarization rather than depolarization in these cells³. Nevertheless there is clear evidence that hormones and neurotransmitters can increase unidirectional Ca^{2+} flux into, for example, pancreatic acinar cells⁴.

The mechanisms underlying this receptor operated Ca^{2+} entry are at present obscure although a link with the increased turnover of phospholipids after stimulation has been suspected for more than ten years⁵ with evidence for such a link being particularly clear in relation to Ca^{2+} entry in blowfly salivary glands⁶. A recently formulated hypothesis⁷ suggests that receptor operated Ca^{2+} entry is secondary to the emptying of an intracellular Ca^{2+} pool mediated by the action of inositol trisphosphate (IP_3) on an electrogenic Ca^{2+} pathway (channel) in the ER^{8,9}. The well-established biphasic nature of agonist-activated Ca^{2+} mobilization is, according to the new hypothesis, explained by an initial emptying of the intracellular (ER) Ca^{2+} pool by IP_3 followed by entry of extracellular Ca^{2+} into the pool and, in the continued presence of IP_3 , into the cytosol. On removal of the stimulant, IP_3 is rapidly broken down closing the Ca^{2+} channel in the ER and Ca^{2+} entry from the outside continues only until the content of the ER pool has reached a level that prevents further entry.

In this model⁷ intracellular Ca^{2+} release and Ca^{2+} entry are both controlled by the same messenger, IP_3 . Ca^{2+} entry is therefore not directly linked to the hormone receptor, but is messenger-mediated. A recent patch-clamp study on pancreatic acinar cells¹⁰ demonstrating an external Ca^{2+} requirement for sustained K^+ channel (Ca^{2+} -activated) opening evoked by a peptide belonging to the cholecystikinin-gastrin group may be relevant in this context since the Ca^{2+} -requirement could be localized at a site separated from the hormone-receptor interaction indicating messenger-mediated rather than receptor-mediated Ca^{2+} uptake. These recent findings¹⁰ appear to be compatible with the hypothesis forwarded by Putney⁷.

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Beam balance test of weak equivalence principle

SIR—In the recent article by Fischbach *et al.*¹, commenting on a *News and Views* piece², a new hypercharge force is postulated as being responsible for various apparently anomalous effects in physics. Although there may not be unanimity in the interpretation of the Eötvös data in terms of this new force³, its existence would have such far reaching consequences in physics that further experiments are clearly required. As suggested by Fischbach *et al.*, further Eötvös-type or galilean experiments come to mind. Here we propose an experiment using a common balance which would allow the force, if it exists, to be measured with good precision. The experiment would be as follows.

The weights of the two test masses made of materials having different values of (B/μ) (see ref. 1 for notation) are compared using a beam balance at two different sites. The first site is in a tunnel deep inside a mountain where it can be assured that there are some kilometres of solid rock in all directions. Here, in the absence of any net hypercharge force, the test masses are adjusted to have apparently equal masses as measured using the balance. The balance and test masses are then moved out of the tunnel to a second site on the surface of the Earth, preferably

at the same altitude and not too distant. According to Fischbach *et al.* we would expect to find at this second site a difference in apparent weight ΔW :

$$\Delta W = a Mg \left(\frac{B_1}{\mu_1} - \frac{B_2}{\mu_2} \right) \quad (1)$$

Taking a value for a derived from the Eötvös data as given in ref. 1 and masses of one kilogram, the largest value of ΔW that could reasonably be obtained would be for masses of an appropriate stainless steel and lithium hydride (^6LiH) respectively, for which $\Delta W \approx 2.5 \times 10^{-7}$ N, equivalent to a 25 μg difference in apparent mass. Replacing the ^6LiH by beryllium would reduce the apparent mass difference to 14 μg but would avoid possible dangers involved in the handling of kilogram quantities of ^6LiH . If, instead, we take the value of a deduced from the geophysical measurements of G , we find a value of ΔW equivalent to 1.5 μg and 0.9 μg for ^6LiH and Be respectively.

The practicality of carrying out such an experiment with a worthwhile accuracy requires first a transportable balance having a reproducibility better than 1 part in 10^9 (equivalent to 1 μg for a 1 kg mass). Traditional knife-edge balances, although there are at least two which achieve such reproducibility^{4,5}, are certainly not transportable so would not be suitable. Such balances are much too delicate and require extreme care in the mounting, handling, and adjustment of the beam and knife edges. However, a flexure-strip balance has recently been described⁶ that is much less subject to these constraints. The beam and flexure elements are designed to be assembled without any adjustment and to be relatively robust. Preliminary results indicate that a reproducibility of 5 parts in 10^{10} can be achieved in the comparison of 1 kg masses. A similar performance may eventually also be possible using a hydrostatic balance⁷. Second, we need to be able to construct pairs of appropriate test masses that are not subject to significant differences in air buoyancy or sorption effects. Adequate test masses could be made by enclosing up to about 1.5 kg of the test material (^6LiH , Be and stainless steel) in stainless-steel vessels made to have nearly identical external volumes and surface finishes. The stainless steel should be chosen to be one of those already used for stainless-steel mass standards, such as Immaculate V, Nicral D or an equivalent AISI 310 steel, whose magnetic and other properties are suitable. Provided that the external volumes of the test masses differ by only a few parts in 10^4 , the differences in their respective air-buoyancy corrections are sufficiently small to be measurable without significant error. Vacuum weighing would of course remove this problem altogether but would, perhaps, introduce further problems due to desorption. It is not difficult to

ensure that the values of the masses themselves and the heights of their centres of mass are sufficiently close for the demands placed on the knowledge of g and its vertical gradient to be modest.

Although the quickest tests of the ideas of Fischbach *et al.* could probably be made by repeating the Eötvös experiment using already existing equipment¹, we think it worth while pointing out that an experiment made using a beam balance could give results of ample precision. Indeed, if the force predicted is shown to exist its presence would, among many other things, lead to modifications in high-precision weighing procedures, to say nothing of its implications for the present definition of the kilogram.

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Blym clone could be contaminant

SIR—In their reply to my recent paper¹ Cooper and Diamond² raise two points—first, that some of the clones I describe are contaminated by the original *HuBlym*-1 clone and, second, that the results of a single transfection assay, not described in my paper, conflict with other assays reported in the paper.

The original clone described in the paper is one of several isolated and designated as non-transforming (using the transfection assay) and reported by Diamond *et al.*³. There has never been any suggestion that this clone, which has the same sequence as *HuBlym*-1, is a contaminant.

It is not possible to exclude the possibility raised by Cooper and Diamond that the other clones described in this paper are indeed contaminants. Unfortunately, the clones, which I left in the laboratory, were disposed after my departure and before the issue of contamination was raised with me. These clones had, however, been designated as transforming or non-transforming in transfection assays read by Cooper and it was precisely because of this classification that they were subsequently selected for sequencing.

Cooper and Diamond cite a transfection assay performed by me in May 1984, when the sequencing of the Burkitt's clones was 90 per cent complete and the cloning and selection of the gene from normal human embryo fibroblast DNA

had just started. This particular transfection was carried out at a time when the assay was not working reproducibly and was an exercise in trying to find out what the problem might be. Therefore this result, which conflicted with several other assays carried out previously and read by Cooper, cannot be considered in any way accurate or meaningful.

It is gratifying that Cooper and Diamond confirm my conclusion regarding the nucleotide sequences of *HuBlym*-1.

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Writing enzyme units in the correct way

SIR—There seems to be a great deal of ambiguity and a frequent scientific error in the literature when the specific activity of enzymes is reported. Very often, it is expressed as “nmol/min/mg”, as “nmol/min per mg” or as “nmol per min per mg”. (See, as examples, refs 1–7, but many others could be cited.) Phonetically it seems correct but I believe it to be mathematically incorrect. It should be expressed as “nmol $\times$ min⁻¹ $\times$ mg⁻¹”.

The expression “ $a/b/c$ ” is indeed ambiguous, because

$$(a/b)/c = \frac{a/b}{c} = \frac{a}{b \times c}$$

$$\text{and } a/(b/c) = \frac{a}{b/c} = \frac{a \times c}{b}$$

therefore, in the linear form “ $a/b/c$ ”, the use of parentheses is obligatory.

To express the term

$$\frac{a}{b \times c}$$

it is necessary to write $a \times b^{-1} \times c^{-1}$ or $a/(b \times c)$.

And similarly, to express the term

$$\frac{a \times c}{b}$$

we must write $a \times c \times b^{-1}$ or $a/(b/c)$.

The formula that is derived for enzyme activity is defined correctly as “nmol $\times$ min⁻¹ $\times$ mg⁻¹” or as “nmol/(min $\times$ mg)”. The common expression “nmol/min/mg” is therefore ambiguous.

The same holds true for other units expressed in a similar formal way, such as mutations/cell/hour and disintegrations/min/tube. I urge the adoption of the correct designation for specific activity of enzymes, and similar units.

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Gamma-ray spectrum of Chernobyl fallout

SIR—The activity in Oxfordshire due to the plume of radioactive material released from the Chernobyl reactor reached a peak on 2 May¹. On 8 May we examined the 'Polyfoam' air filters routinely used in our laboratory air supply system and monitored a small but distinct amount of β - and γ -radiation activity. We have measured the γ -ray energy spectrum from the filters using a high-efficiency (40%) germanium detector, shielded from room background.

Figure 1 shows the complex γ -ray spectrum from the filters. The background is due to Compton-scattered γ -rays, which do not deposit their full energy in the detector. An analysis of the full-energy peaks yields a definite identification of 16 radioisotopes, with a further three (weaker) tentatively identified radioisotopes. The activity of each radioisotope is categorized as strong or weak in Table 1. The relative activities for the most prominent isotopes vary significantly from those deduced by Fry *et al.*¹, which serves to emphasise that these coarse filters were not designed to collect fallout products. The measured activity of $\sim 2,000$ Bq m⁻² of ¹³¹I on each 0.1m² filter suggests that $\sim 1\%$ of the local iodine activity¹ was deposited by the air flow of

Table 1 Relative γ -ray activities of radioisotopes measured in laboratory air filters

Isotope	Relative activity*	Half-life
¹³¹ I	S	8 d†
¹³⁷ Cs	S	30 yr
¹³² Te	S	3 d
¹³² I	S	2 h
¹⁰³ Ru	S	39 d
¹³⁴ Cs	S	32 yr
¹⁰⁶ Ru	S	367 d
¹⁴⁰ Ba – ¹⁴⁰ La	S	13 d–40 h
¹³⁶ Cs	W	13 d
⁹⁵ Zr – ⁹⁵ Nb	W	64–35 d
¹⁴¹ Ce	W	33 d
⁹⁹ Mo	W	3 d
¹⁴⁴ Ce	W	284 d
¹²⁹ Te ^m	W	33 d
[¹²⁷ Sb]	[W]	4 d
[¹⁰⁸ Rh]	[W]	1.5 d
[¹⁴³ Ce]	[W]	1.5 d

*Identified radioisotopes are listed in order of activity. Isotopes with activities greater than 10% that of ¹³¹I are labelled strong (S) and those with activities less than 5% are labelled weak (W). † Days.

2×10^4 m³ per day.

All of the identified radioisotopes are ²³⁵U fission decay products. The criteria for observing a particular fission product in such a simple γ -decay experiment are: (1) the feeding mass chain should have a lifetime ≥ 1 day and ≤ 500 yr; (2) the fission yield should be $\geq 0.5\%$ for the mass chain; (3) the β -decays should significantly populate excited nuclear states which subsequently γ -decay. Using these simple criteria, we find that all of the expected mass chains are accounted for,

except masses 133 and 147, whose identifications are made difficult by the complexity of the spectrum.

Most of the activity (70%) will decay over the next month and the levels will approach those measured normally from the natural thorium and radium background. Air-supply filters with greater air flows or greater capture efficiency will have proportionally greater activity.

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Q1146+111B,C quasar pair: illusion or delusion?

SIR—The quasar pair Q1146+111B,C¹ has been re-observed by Turner *et al.*² who argue that it comprises two gravitationally lensed images of a single source. In this letter, we show that we are probably observing two distinct though neighbouring quasars.

Were quasars placed randomly on the sky, the probability of finding another quasar of apparent magnitude m within an angle θ of a given quasar would be $\propto P_c(\theta, m) \approx n\pi\theta^2$, where $n(m)$ is the sky density of quasars brighter than magnitude m . For Q1146+111B,C, $\theta = 157$ arc s, $n(18.5) \sim 1$ per deg² (ref. 4), giving $P_c \sim 6 \times 10^{-3}$. However, quasars are probably not distributed uniformly throughout the Universe, and there is evidence that they are clustered in much the same way as galaxies^{5,6} (but see ref. 7), so their two-point correlation function is $\xi(r) \approx (r/r_0)^{-1.8}$, where $r_0 = 5(1+z)^{-1}h^{-1}$ Mpc, h is the Hubble constant $H_0/100$ km s⁻¹ Mpc⁻¹ and the cosmological density parameter $\Omega_0 = 1$. The probability associated with this excess for a given quasar to have a companion within angle θ brighter than magnitude m is then

$$P_c(\theta, m, z) = 2\pi(1+z)^3 \int_0^{\theta} db b \times \int_{-\infty}^{\infty} dL \Phi[\xi(b^2 + L)^{1/2}] \quad (1)$$

where $D(z)$ is the angular diameter distance of the quasar, and $\Phi(L, z)$ is the integral luminosity function for quasars with luminosity $L(m, z)$. Now, $\Phi(L, z) = (dn/dz)/D^2(1+z)^{1/2}$ (ref. 8) and observations⁴ indicate that for $0.5 < z < 2$, quasars of a given magnitude are uniformly distributed in redshift with $dn/dz \approx 0.5n$. Substitution in equation (1) then gives $P_c(\theta, m, z) = 38(1+z)(1+z-z-1)\theta^3\xi(\theta D)dn/dz$ for $D\theta \leq 2r_0$, where n is per steradian and θ in radians. Numerically, $P_c \sim 2 \times 10^{-4}$.

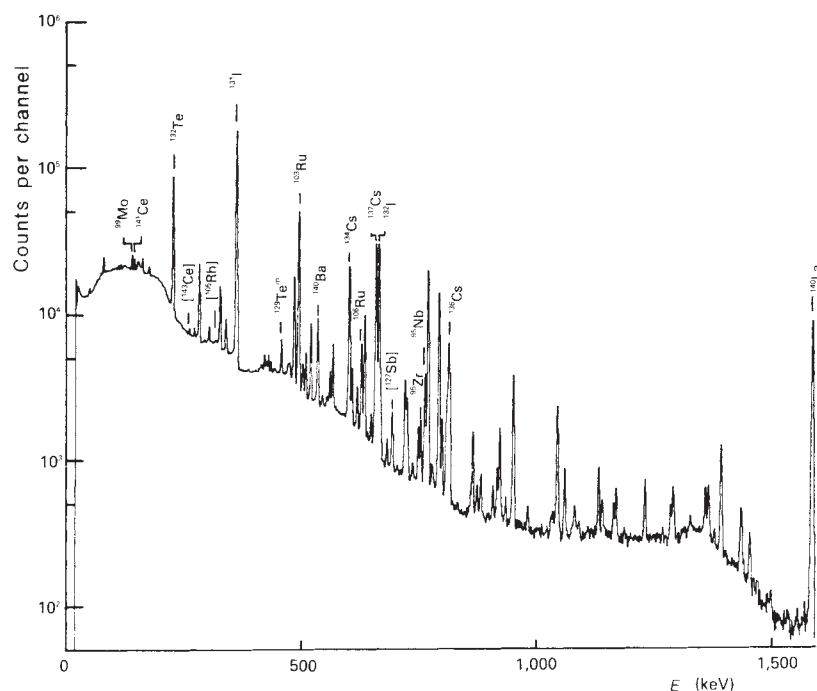


Fig. 1 Part of the γ -ray spectrum obtained with a Ge(Li) detector from a typical air filter. The strongest identified peak of each radioisotope is labelled.

Published quasar catalogues^{9,10} contain $N(19) \sim 2,000$ quasars brighter than $B = 19$. Unpublished surveys probably treble this number. The expected number of random associations with the separation and magnitude of Q1146+111B,C is $NP_c(157 \text{ arc s}, 18.5)/2 \sim 20$, which is roughly what is observed^{5,9}. The expected number of closely clustered companions is $NP_c(157 \text{ arc s}, 18.5, 1.0)/2 \sim 1$. Hence we would expect by now to have discovered of the order of one physically associated pair of magnitude 18.5 quasars separated in angle by less than 157 arc s and in velocity by the velocity dispersion of small groups which dominate the correlation function, $\sim 300 \text{ km s}^{-1}$. We should not be unduly surprised if Q1146+111B,C fails tests of the lensing hypothesis¹¹ — it may be that expected pair.

In conclusion, we suggest that the necessity for lensing in some of the other gravitational lens candidates should be re-examined. This is especially important for faint quasars when there is no independent evidence for lensing (for example, an intervening giant galaxy or distortion of background galaxies) and no sign of common spectroscopic or morphological peculiarities. (Overall spectral dissimilarity need not rule out lensing because quasars can vary significantly in the time delay between two images.) The quasar pairs Q2345+007A,B (ref. 12) and Q1635+267A,B (ref. 13) in which the fainter quasars are both roughly magnitude 21 both give $P_c \approx P_r \sim 2 \times 10^{-4}$, if we assume that the correlation function is valid for proper distances $\sim 20 \text{ kpc}$ (as it is for the galaxy-galaxy correlation¹⁴; it has been suggested that the quasar-galaxy correlation function is even higher^{15,16}). It is therefore not unreasonable that these two pairs comprise distinct objects; since $P_r \sim P_c$ we should expect to find a few similarly separated quasars with discrepant redshifts, for example, Q1548+114A,B (ref. 17).

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Beware the cups that cheer

SIR—Aluminium toxicity, intoxication or accumulation is associated (not necessarily causally) with several conditions including dialysis encephalopathy¹ and osteomalacia², the amyotrophic lateral sclerosis of Guam, and Alzheimer's disease³ (believed to account for some one third of a million UK sufferers from senile dementia and said to affect over two million people in the United States). Edwardson and his colleagues have recently shown⁴ the presence of aluminosilicates in senile plaques in Alzheimer's disease. Dietary intake of aluminium has not generally been regarded as a hazard, though one Swedish study⁵ showed that cooking rhubarb in aluminium saucepans mobilized a significant amount of aluminium, presumably complexed by oxalate (ethanedioate). We would like to suggest that attention is paid to the high aluminium content of tea leaves and their infusion.

The tea bush (formerly *Thea sinensis*, now *Camellia sinensis*) is grown on acid soil, and tolerates high levels of aluminium, accumulating great quantities⁶ of it. Indeed, potassium alum is used as a fertilizer⁷. The only other food plant which tolerates such high aluminium levels is the cranberry. The tea bush accumulates aluminium in the leaves to enable it to overcome a high level of aluminium in the leached acid soils in which it thrives.

We have examined samples of Chinese, Indian and Russian tea bought as packets from a local supermarket. The X-ray powder picture of each dry ash (all of surprisingly good quality) was essentially that of α -alumina (Al_2O_3). Other weak lines were present. The aluminium content (by gravimetric analysis) of these dry ashes varied from 8,700 p.p.m. to 23,000 p.p.m., in line with several reported analyses; up to 20,000 p.p.m. Al/dry weight have been reported in mature leaves⁸.

We infused the teas in a teapot for up to 30 minutes using Cardiff tap-water (very soft), then analysed the infused liquids for aluminium with gravimetric references to underpin routine atomic absorption meas-

urements. A typical finding was that the infusion of Russian tea had about 100 p.p.m. aluminium (100 mg dm^{-3}), and the Indian and Chinese typically had 40 and 60 p.p.m. aluminium, respectively.

Since many Britons drink a litre of tea a day, tea is likely to be as great a source of aluminium as any other in the British diet. (The aluminium foil often used for lining tea chests seems unlikely to exacerbate the problem).

Assuming (in the absence of information on the transfer of aluminium from diet into organs) that any source of aluminium is to be avoided at least for those suffering from conditions related to high aluminium levels, the belief that tea is a sovereign remedy under all circumstances may need revision. "The cups that cheer but not inebriate", may not be as true as William Cowper thought in 1785.

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Frequency of dizygotic twinning

SIR—I am puzzled by a calculation in J.M. Diamond's article "Variation in human testis size" (*Nature* **320**, 488; 1986). In it he states that a single ovulation with intercourse will result in a live birth with probability $1/4$ and therefore a double ovulation will result in dizygotic twins with probability $1/16$. He concludes that the double ovulation frequency must be 16 times the observed dizygotic twinning rate among births.

This conclusion is incorrect since a large fraction of the double ovulations will not lead to any birth at all. A double ovulation will yield zero births with probability $9/16$, one birth with probability $6/16$ and two births with probability $1/16$. Hence the probability that a double ovulation will yield twins conditioned on there being any birth at all is $(1/16)/(1/16+6/16)=1/7$. Therefore Diamond should conclude that the double ovulation frequency is 7 times the observed twinning rate. Thus, for example, the Yoruba women would have a double ovulation frequency of 34.3% rather than 78%, as stated.

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All around biotechnology

A.N. Emery and A. Lyddiatt

Comprehensive Biotechnology: The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine.

Editor-in-chief Murray Moo-Young.

Pergamon:1985. Four volumes, pp.3,764. £695, \$995.

BIOTECHNOLOGY feeds upon many disciplines, and is dependent upon the cross-fertilization of ideas and information from a wide variety of sources. Ready access to the relevant literature is frequently inhibited by its style and format, and it is this drawback that is addressed by *Comprehensive Biotechnology*.

The work has been promoted as an integrated presentation of the basic principles, methods and applications of biotechnology. This is a formidable claim, and the fact that four volumes totalling over 3,500 pages were found to be necessary is a measure of the difficulty of the task that the editors set themselves. Moreover, the editorial board, under the direction of Murray Moo-Young, set out to assemble the work under several self-imposed constraints: they had to coordinate successfully an international team of 150 writers, combine intellectual appeal with comprehensive coverage and publish all four volumes at the same time. Such testing goals have been largely met in a work which lives up to its billing.

The four volumes are respectively entitled *Scientific Fundamentals*, *Engineering Considerations*, *Current Commodity Products* and *Speciality Products and Service Activities*, and each of them is in turn subdivided into either two or three sections. Volume 1, Section 1 covers genetic and biological fundamentals (including isolation methods, nutrition, growth and death of microorganisms as well as their genetic manipulation), while the regulation of cellular metabolism and enzyme evolution, mechanisms and kinetics are dealt with in Section 2. The introduction rightly describes this as a "pedagogic academic coverage" of the scientific disciplines underpinning the field as a whole, but the absence of stuffiness in the prose or inhibitory jargon is a strong incentive for the reader to explore the text rather than merely use it for reference. The relatively low-key coverage of animal and plant cell culture (combined in one lone chapter), however, reflects an emphasis out of keeping with the rapid increase of activity in these areas.

Volume 2 contains sections on bioreactor design, operation and control, and on upstream and downstream processing, written by a varied group of academic and industrial authors. This mix is not wholly successful, because some of the contri-

butors have been allowed to reflect rather too strongly their commercial affiliations. Also, a stricter editorial hand would have avoided the repetition found, for example, in all four chapters on instrumentation, data handling and control, as well as in four chapters on aspects of membrane-based processes. Cross-referencing both between operations described within Vol. 2, and with applications in Vol. 3, would have greatly added to the value of these two components of the work.

Volumes 3 and 4 are described in their introductions as "utilitarian practical account[s] of commercial processes and products". The authorship is truly international (except for Soviet and Eastern European representation), and is largely drawn from industry. Volume 3 gives good accounts of present practices in the development and manufacture of health-care products, food and beverage products, and industrial chemicals, biochemicals and fuels. Volume 4 is much more ill-assorted: the first section, in particular, while aiming to introduce potential applications in biomedicine and chemotherapy, agriculture and other areas, is also left to pick up pieces of instrumentation, detection and containment of biohazards, and even surface thermodynamics. The remaining two sections give, first, an interesting and helpful international comparison of governmental approaches to both support and regulation of biotechnology, and lastly a useful blend of the

theory and practice of waste management and pollution control.

It would need a football team of specialists to provide a detailed evaluation of the diverse contents of these four volumes. Instead, we sought to assess *Comprehensive Biotechnology* by making it available in our laboratories for anyone who cared to consult it — academic and research staff, students and industrial collaborators with interests across a wide range of subjects. By and large, their response has been enthusiastic. Specialists have generally judged the treatments of their own areas to be thorough, well illustrated, topical and generously referenced (up to about mid-1983). Venturing into less-familiar areas, we and our colleagues have found ourselves neither patronized by too shallow a text nor left floundering, bereft of explanation or definition. Each volume includes a glossary of terms and is individually indexed, though only Vol. 4 contains a cumulative index to the work as a whole — indeed, a general lack of inter-volume cross-referencing is a weakness. There is surely a strong case for producing a separate cumulative index.

Our personal expertise is in biochemical engineering, so it is inevitable that our more detailed criticisms arise with Vol. 2, and particularly the section on upstream and downstream processing. Here there are some surprising omissions, for example protein precipitation, non-specific adsorption, evaporation and drying. Such operations are now widely used in biochemical recovery, and absence of discussion of them is puzzling. This, however, could well be rectified in the subsequent, "update" volumes which the editors promise will appear at intervals to chronicle the scientific and technical developments of the future. Inclusion of an overview of the integration of unit processes in production and recovery operations would be beneficial, as would coverage of topics such as multiphase mixing,

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Bacterial change — a phase-contrast photomicrograph of spores and vegetative cells of Bacillus fastidiosus. The picture is reproduced from Developmental Biology of the Bacteria, by Martin Dworkin, published by Benjamin/Cummings. Price is \$31.95, £24.95.

S.C. Holt

biosensors, product polishing and quality control.

Finally, we have doubts about the grouping of certain topics and (more importantly) the physical robustness of the volumes. This is unquestionably a work of reference which will have to endure the rigours of usage in both library and laboratory. Two of the books contain over 1,100 pages in bindings likely to surrender to heavy patronage. Division into eight volumes might have inflated production costs but would have guaranteed a prolonged physical life — surely something to be expected from a work costing nearly £700. It would also have permitted a more rational allocation of material, avoiding the uneasy juxtapositions of topics in Vols 2 and 4, and consolidating individual volumes as entities in their own right.

For all that, Murray Moo-Young and his colleagues have brought off a notable success in producing this work and our limited criticism does not diminish this overall view. *Comprehensive Biotechnology* will be an essential purchase for all departments and institutions, academic or industrial, that claim an interest in any aspect of the ill-defined field popularly known as biotechnology. □

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Controlling disease in the valleys

G. Webbe

Schistosomiasis: The St Lucia Project. By Peter Jordan. Cambridge University Press: 1985. Pp. 442. £35, \$49.50.

THE aim of the St Lucia schistosomiasis project was to compare the effects of control of the snail intermediate host, chemotherapy (that is, drug treatment) and improving water supplies in reducing transmission of *Schistosoma mansoni*, and thus the incidence, prevalence and intensity of the infection. The project ran from 1966 to 1981. It was based upon an epidemiological model which predicted the relative importance of the three approaches to control, and the experimental programmes were therefore designed for maximum impact in separate valleys which had similar epidemiological characteristics. These programmes are described in Part I of Peter Jordan's book.

In one valley, area-wide mollusciciding was directed against snail colonies, detailed research into the dynamics of transmission having been undertaken as a basis for an appropriate snail control strategy. In a second valley, the contamination with schistosome ova was reduced by chemotherapy; treatment was offered to anyone found to be infected at any of the four annual surveys. Water supplies were provided in part of a third valley to reduce exposure to infection in rivers and streams. Piped water was introduced to individual houses in five valleys, together with communal laundries with shower units, and simple pools for recreational purposes.

After four years the effects on transmission of *S. mansoni* through snail control and the improvement of water supplies had been assessed, and chemotherapy was offered to people found to be infected. At this time, too, the area-wide strategy of snail control was changed to one of focal control. Over the next four years different consolidation strategies after therapy were tried out in each of the valleys. Further control schemes (through the provision of latrines or chemotherapy) were also initiated to compare the effect on transmission of reduced contamination with *S. mansoni* ova.

Comparison of the control strategies, based on parasitological findings, showed that the chemotherapy programme resulted in the cheapest, quickest and greatest fall in incidence, prevalence and contamination potential (cost \$1.00 per person per year). In the snail control schemes, where area-wide mollusciciding and focal control were applied, the overall declines in incidence and prevalence were

similar, with intensity being reduced more with area-wide control (cost \$3.24 and \$2.87 per person per year, respectively). There was substantial reduction in all indices of infection in the water supplies programme (cost \$2.99 per person per year). The results of additional chemotherapy were disappointing, however, while the effect of the provision of latrines remained inconclusive because of sporadic water supplies. In the maintenance phase, after chemotherapy in all areas, the best results came from focal snail control or the provision of individual household water supplies and communal laundries and showers.

The overall results of the St Lucia project confirmed the predicted relationship between morbidity and intensity of infection, and it seems that, for rapid disease prevention, chemotherapy will now be the main component of schistosomiasis control. The effectiveness of any drug delivery system must, however, determine the level of residual infection after treatment in a particular area. Moreover, in global terms, the transmission of schistosomiasis is characterized by its variability. While patterns of human contact with water, as well as human-parasite-disease relationships, are similar around the world, the transmission patterns differ greatly according to the ecology of the species of snail concerned. Thus, while it highlighted the relative cost-effectiveness of different control approaches over a limited period, the St Lucia project does not provide a basic protocol which can be applied in all situations. Further, more information is required about different chemotherapy regimens and even about the effects of treatment on acquired immunity, before formulating treatment policy. With such a limited number of effective compounds available, the problem of drug resistance must also be considered when developing long-term maintenance strategies requiring periodic re-treatment campaigns.

Part II of the book contains details of several related studies that were carried out in St Lucia. Among these, the accounts of epidemiological research on human exposure, morbidity and the longevity of adult worms, and of the immunological work, are of special interest.

The book is well illustrated and the data are very clearly presented. Altogether it is an important contribution to the literature on schistosomiasis; it strongly reflects the multidisciplinary nature of the St Lucia project, and it contains a unique wealth of information for parasitologists and all who are concerned with control of this disease. □

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Issues of analysis

Paul H. Harvey

Ecology and Natural History of Desert Lizards. By Eric R. Pianka. *Princeton University Press*: 1986. Pp. 208. Hbk \$45, £30; pbk \$19.95, £13.30.

FOR the past 20 years Eric Pianka has compared desert lizard communities in America, Africa and Australia, using standardized methods to allow comparisons among study sites within and between continents. The main results of his work are now brought together in a book which is not short on data; in addition to 31 pages of appendices, over 60 pages of the 150-page text are taken up by tables and figures.

Most of the book describes differences in reproductive tactics, activity and feeding habits of the various species, anatomical correlates of ecology, and community structure. The discussions are frequently based on statistical and multivariate summaries of the data. Such analyses can be dangerous, because hidden assumptions often lead to incorrect conclusions. Over the past ten years, there has been particular focus on this issue and Pianka is acutely aware of the pitfalls involved. Indeed, he devotes a full chapter to describing and assessing the methods used in the analysis of community structure. Most of his conclusions seem sound; for example, using a carefully designed "computer transplant experiment" Pianka and Larry Lawlor show that diets of particular species tend to change from site to site in a way that complements the diets of other species with which they are found.

Not all the analyses are as careful as this, however. At one point Pianka identifies what he terms a "transcendent" relationship. Some lizard species thermoregulate more effectively than others. The extent to which lizards thermoregulate can be described by their change in body temperature over a set range of air temperatures. But, at a set air temperature, some lizards are warmer than others. Pianka believes that these two components of the relationship between body and air temperature are not independent of each other, and plots the slopes relating body temperature to air temperature against their respective regression intercepts for a sample of 82 lizard species. He claims that the resulting highly significant negative relationship "represents an innate design constraint imposed by lizard physiology and metabolism". But, for given air and body temperatures, slopes and intercepts are not *expected* to be independent values: if we change the slope, we also change the intercept.

We clearly need to compare Pianka's data with a model in which slopes are in-

dependent of the air and body temperatures measured. To set up such a model, I randomly shuffled the empirical slopes among the different species and then calculated new intercepts using the mean external and body temperatures for each species. I repeated this procedure ten times and the correlation between slope and intercept ranged between -0.87 and -0.90, which to some extent describes Pianka's "transcendent" relationship. Pianka's relationship between slope and intercept may be tighter than expected, but he has not demonstrated that it is under a clearly defined null hypothesis. Indeed, his description leads the reader to assume that the expected correlation between slope and intercept is zero.

One chapter deals with reproductive tactics and, here again, I suspect that statistical analyses have missed their mark.

Body size effects are either ignored or "removed" by taking simple weight ratios. Since larger species of most vertebrate groups are known to lay disproportionately small eggs (the relationship is one of negative allometry), Pianka's techniques are probably inappropriate and his analyses need to be repeated correctly.

Nonetheless, the book has considerable strengths. It is a rich source of unique data; it highlights our ignorance of factors influencing the structure of even relatively simple communities; and it documents excellent natural history observations of particular species. In addition, the text is enlivened by a delightful collection of superbly reproduced colour photographs illustrating the variety of species studied. □

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Technique in time

D.J. Huntley

Thermoluminescence Dating. By M.J. Aitken. *Academic*: 1985. Pp. 359. Hbk £50, \$59; pbk £30, \$34.95.

MARTIN Aitken's first book, *Physics and Archaeology*, published in 1961, contained only a page on thermoluminescence dating. This new book presents a stunning contrast.

In 1961 it was thought that thermoluminescence (TL) resulted from radiation damage due to α -particles emitted by naturally occurring uranium and thorium; we know now that it results from electrons becoming trapped at crystal defects as a consequence of their liberation by various kinds of radiation. The general idea of using TL to determine past radiation doses, and hence the ages of samples (using separate measurements of the dose rates), has been around for several decades. It was not, however, until Aitken took up the subject that real progress was made and reliable dates of practical value to archaeologists were obtained. Most advances in the technique originated in Aitken's own laboratory at Oxford, but description of them is spread over such a wide literature that we all welcome having them brought together in a single book.

In the first two chapters the basic principles and methods are outlined in language that anyone with a scientific training should be able to follow. Thereafter the reader will learn that the subject is riddled with complications. The archaeologist who has studied ^{14}C dating knows that here one has to measure only the $^{14}\text{C}/^{12}\text{C}$ ratio in order to calculate a date. In TL dating, on the other hand, the number and complexity of the problems that have to be taken into account is extraordinary. Non-linear

TL response, differing TL sensitivities, inhomogeneous samples, varying water contents, radon escape and a host of other factors have to be coped with, and all of them are discussed in detail by Aitken. Some of these complexities can, however, be thought of as simply reflecting the great richness of TL dating, which now subsumes several different techniques (fine-grain, quartz inclusion, subtraction, pre-dose, zircon, etc.). Again, all are described thoroughly. Each chapter includes a page or more of "Technical Notes", where many useful details are given without interrupting the main text, while the book itself ends with 13 appendices, taking up 90 pages. These are an invaluable reference source which TL workers will often have cause to consult.

The shortcomings of the book are minor. Aitken admits to being biased towards research done at Oxford; here the book is naturally strong, but in consequence work done elsewhere receives less thorough treatment. My main criticism, however, is reserved for a lack of rigour at some points and for Aitken's use of units. An "ionized electron" (p.42) is not part of my vocabulary, and reference to the activity of potassium as 89.5 per cent beta and 10.5 per cent gamma will confuse the unwary. "Annual dose" is used where "dose-rate" is meant, R is used for α -particle range in units of length on p.299 and units of mass per unit area on p.300, and a sievert can no more equal a gray than a digested apple can equal a fresh one. These and others like them are not too serious, though they will puzzle those not sure of their science.

This is a book by a physicist for physicists, and an essential acquisition for any TL dating laboratory and library. It will be the standard reference for many years. □

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Ways to dispose of radioactive waste

J.L. Knill

Geology and Radwaste. By A.G. Milnes. Academic: 1985. Pp.328. Hbk £50, \$60; pbk £36.95, \$39.95.

EVENTS during the first months of 1986 have placed the nuclear industry in Britain at the centre of public attention. A series of minor incidents at the Sellafield nuclear fuel reprocessing plant, the identification of four possible sites for the shallow disposal of low-level radioactive waste, the publication of the Environment Select Committee's critical report on radioactive waste — all have been greeted with outcries from the various groups opposed to all forms of nuclear proliferation. Now, of course, we have had Chernobyl, and the attacks on the nuclear industry will intensify.

The consistent reply from the industry has been that its critics are over-reacting, that technical improvements will continue to be made and that the risks associated with release of nuclides into the environment are overstated. These differences of opinion will not be resolved easily, and will fuel the continuing debate on the sources, and disposal methods, of radioactive waste.

Such waste is traditionally subdivided into four classes: very low, low, intermediate and high-level/heat-generating. However, this classification can be over-simple in that short-lived, intermediate-level waste may be disposed of together with low-level waste, and some argue that α -emitting low-level waste should be grouped with intermediate-level waste.

Because of the long half-lives of the radionuclides it contains, and the daughter products, high-level/heat-generating material will be an environmental hazard for hundreds of thousands of years. For this reason, disposal must be at depth in a geologically stable environment. A British research programme into deep disposal was stopped by the government in 1981, and for the next 50 years high-level waste will be stored on the surface as a fluid, and progressively encapsulated in glass, in order to maximize dissipation of the heat which is less easy to control underground. Deep disposal will then follow.

Since 1981 the responsibility for finding, proving and developing low- and intermediate-level waste disposal sites has rested with UK NIREX Ltd, a company controlled by the energy and nuclear industry. Selection of a deep site for intermediate-level waste in an anhydrite mine at Billingham led to local opposition, and then withdrawal of the site by the government. The choice of Elstow for an engineered low-level disposal site in clay met a similar reaction but, now, three further shallow sites in clay have been named; after detailed investigation, one of them will be selected and the case for development put to a public enquiry.

Against this background, the appearance of *Geology and Radwaste* is timely. Milnes defines his reasons for writing the book in the preface. He notes that the geological uncertainties are inherent, that simple solutions rarely exist and that this field offers much scope for conflict, as did the classic geological controversies of the past. Despite his conviction that the nuclear road is not the one we should take, he recognizes that here is a problem that will not go away, and that radioactive waste disposal is no more of an environmental hazard than the uncontrolled emission of (for example) carbon dioxide, sulphur dioxide and heavy metals.

The book is divided into three parts, the opening chapter of Part I providing a summary of radioactive waste types. As in

the remainder of the book, the text here is exceptionally clearly written and well illustrated, meeting the author's objective of being accessible to a wider audience than the specialists involved. Also notable is Milnes's impressive grasp of the literature; much that is published on this subject is unrefereed, and is available only in rather obscure reports and conference proceedings. Each chapter ends with a summary of selected literature, with full references at the back of the book.

The second chapter of Part I covers the alternative methods of disposal of low-level liquid wastes, and the disposal of low- and intermediate-level solid wastes at sea, by shallow burial and in deep, mined cavities. The particular problems of high-level waste are highlighted, attention being drawn to some of the more exotic solutions that have been offered in the past.

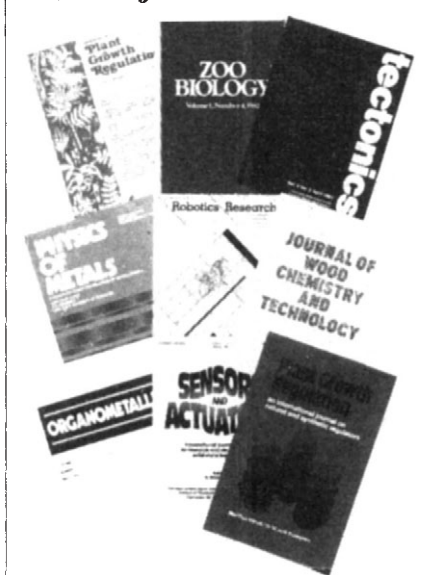
Part II, occupying about two-thirds of the text, gives the geological perspective to radioactive waste disposal by describing the subsurface and surface processes operating through geological time. Much of the material here is familiar, but the author skilfully draws in case histories and examples to illuminate particular points; for instance, rates of denudation are illustrated by reference to the Texas Panhandle and to a mine test site at Mol, in Belgium. The eleven chapters systematically cover the relevant rock-forming, tectonic, geomorphological and fluid processes which provide the potential environments for storage and disposal. These processes are discussed in the context of the time-scale within which hazardous disruption of a repository could occur.

The two final chapters review predictive methods for identifying the disposal system most likely to guarantee that any release to the environment will not exceed regulatory limits. Safety assessment of potential sites demands expertise well beyond geology, and the book does not provide a guide to methods of site selection, site specific investigation or the geological aspects of safety assessment. Within his chosen compass, however, Milnes has produced a lucid and thorough book which should be read by all of those who have involvement with, or interest in, the geological disposal of radioactive waste. In the aftermath of Chernobyl, that is likely to be very many people indeed. □

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• *Management of Toxic and Hazardous Wastes*, published by Lewis Inc., Chelsea, Michigan, contains the (updated) proceedings of a conference on the subject held in Columbus, Ohio, in 1983. Price is \$49.95, £45.95.

New journals review



On 25 September *Nature* will publish the sixth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1986 issue are that:

(i) the first number appeared, or the journal was retitled, between June 1984 and May 1985 (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);

(ii) it is published at least three times a year;

(iii) the main language used is English.

Publishers and learned societies are invited to send four different issues of each suitable periodical, including the first and most recent numbers (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details for both 1986 and 1987 should be included.

Galaxy formation by mock gravity

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Absorbing particles in an isotropic radiation field experience an attractive force due to their mutual shadowing. If the pre-galactic universe contained suitable sources of radiation and absorbing material, an instability caused by this effect may have led to the formation of galaxies and galaxy clusters. Other consequences include the segregation of galaxies from dark matter and a strong anisotropic far-infrared background.

RADIATION pressure can have a significant effect in structuring the interstellar medium, dominating gravity for some species and environments by many orders of magnitude¹⁻⁵. As first pointed out by Spitzer², absorbing particles in an isotropic radiation field experience 'mock gravity', an inverse-square attraction due to their mutual shadowing. Gamow⁶ originally suggested that mock gravity resulting from Compton absorption of the microwave background by free electrons might provide a source of cosmological instability. However, as demonstrated by Field⁷, this effect is negligible, both because matter and radiation are very close to thermodynamic equilibrium, and because the temperature is so low at epochs of interest that scattering exceeds absorption by a very large factor. On the other hand, it is possible that conditions in the pre-galactic universe might resemble those in the interstellar medium today, with stars emitting ultraviolet radiation and dust providing significant continuum opacity⁸⁻¹⁵. In this situation, mock gravity can drive a rapidly growing instability which might extend up to scales comparable to those observed in galaxy clustering.

Several non-gravitational mechanisms have been investigated which could, in principle, generate large-scale structure^{16,17}. Mock gravity provides another such mechanism, but is unique in several respects. No true instability has previously been demonstrated to occur on such large scales. The gravitational instability usually invoked to explain large-scale structure is just a reflection of energy conservation in pre-existing fluctuations which are left behind as the universe expands around them. Mock gravity can behave like a true instability in that a wide range of scales can grow to nonlinearity in much less than an expansion time. It has a natural scale-length (the photon interaction length) which limits the size of structures, and a natural scale of binding energy determined by the radiation field. Together these scales determine the mass and density of the largest structures produced. Segregation of light and dark matter, and of enriched and unenriched gas, are also natural consequences of mock-gravitational instability. Within this theoretical framework, the development of structures qualitatively like those observed is a natural consequence of certain general conditions in the pre-galactic medium, and has little dependence on the previous large-scale mass distribution.

Our analysis differs from previous discussions^{2,3} of mock gravity. Instead of presupposing an isotropic, externally generated radiation field, we introduce a homogeneous density of radiation sources. In linear theory, we are then able to treat the growth of optically thick perturbations. Our analysis includes the expansion of the universe, which was omitted from discussions focusing on the interstellar medium. We make the usual assumptions that the absorbing particles are continuum absorbers such as dust grains, that scattering can be neglected, and that the re-emitted radiation has a negligible interaction with the grains.

Mock gravitational instability

Consider a uniformly radiating background medium with luminosity density l . Within this medium are distributed dust grains with number density n , and frequency-averaged absorp-

tion cross-section σ . The sky brightness in a direction $\mathbf{i}$ seen from the origin is

$$S(\mathbf{i}) = \int_0^\infty dr r^2 (l/4\pi r^2) \exp \left[- \int_0^r dr' n(r') \sigma \right] \quad (1)$$

Decomposing n into a uniform and a perturbed part $n_0 + \delta n$, we find the perturbation in brightness

$$\delta S(\mathbf{i}) = (-l/4\pi) \int_0^\infty d\tau e^{-\tau} \int_0^{\tau/n_0\sigma} dr' [\delta n(r')/n_0] \quad (2)$$

The net force exerted by radiation pressure on a grain at the origin is then

$$\mathbf{F} = -\frac{\sigma}{c} \int d\Omega \delta S(\mathbf{i}) \mathbf{i} = -\frac{l\sigma}{4\pi c} \int_0^\infty d\tau e^{-\tau} \int_0^{\tau/n_0\sigma} d\Omega \frac{\delta n(r') \mathbf{i}}{n_0} \quad (3)$$

Let us consider the force due to a plane-wave perturbation $\delta n/n_0 = \delta_k e^{ik \cdot x}$. Then $\mathbf{F} = F_k e^{ik \cdot x} \mathbf{k}/|k|$ with

$$F_k = -\frac{l\sigma}{c} \frac{\delta_k}{ik} [1 - p^{-1} \tan^{-1} p] \quad (4)$$

where $p = k/n_0\sigma$. For a static medium the equation of motion of such a linearized plane wave is $\ddot{\delta}_k = -ikF_k/m$, where m is the effectively coupled mass per dust grain. Hence

$$\ddot{\delta}_k = \frac{l\sigma}{mc} [1 - p^{-1} \tan^{-1} p] \delta_k \equiv \omega_k^2 \delta_k \quad (5)$$

and the fluctuation grows exponentially with growth rate ω_k . For optically thin perturbations ($p \gg 1$) ω_k is independent of wavelength. In the opposite limit, the growth rate decreases with $\omega_k \propto p$.

In an expanding universe filled with well-coupled dust and gas, the equation of motion becomes

$$\ddot{\delta}_k + (2\dot{a}/a) \dot{\delta}_k = (l\sigma/mc) [1 - p^{-1} \tan^{-1} p] \delta_k \quad (6)$$

where $a(t)$ is the expansion factor, k is a co-moving wavenumber defined so that $p \equiv k/a(t)n_0(t)\sigma$, and gravitational effects have been neglected. The growth rate of the fluctuation relative to the expansion depends on the dimensionless parameter

$$A \equiv (2\omega_k(t)/3H)^2 \equiv A_0 [1 - p^{-1} \tan^{-1} p]; \quad H \equiv \dot{a}/a$$

defined by the coefficients in this equation. For optically thin perturbations in an approximately Einstein-de Sitter universe, $A \equiv A_0 = 4l\sigma/9mcH^2$ is independent of time provided the properties of the dust and the sources do not evolve. In this situation, the fluctuations grow as

$$\delta_k \propto t^\alpha, \quad \alpha = (A_0 + \frac{1}{36})^{1/2} - \frac{1}{6} \quad (7)$$

For large A_0 , $\delta_k \propto t^{\sqrt{A_0}}$ and growth is rapid. For a given perturbation $p \propto a^2$, and even for large A_0 , rapid growth can occur only after a characteristic time $t_c(k)$ defined by $p(t_c, k) = 1$. The equation for δ_k can be written

$$\ddot{\delta}_k + 4\dot{\delta}_k/3t = (A_0/t^2) [1 - (t/t_c)^{-4/3} \tan^{-1}(t/t_c)^{4/3}] \delta_k \quad (8)$$

The solutions of this equation are shown in Fig. 1 for several values of the parameter A_0 . The gravitational mode $\delta_k \propto t^{2/3}$ is also shown for comparison. Rapid growth occurs only after $t = t_c$ and only for $A_0 > 1$. The transition at $t = t_c$ is quite sharp. Note that the initial amplitude of the fluctuation need not result from an enhancement in physical density. Inhomogeneities in the distribution of luminosity sources and of dust production sites will introduce initial white noise fluctuations on all scales greater than those characteristic of these processes. If, however, real density fluctuations are dominant on large scales, mock gravity will take over from normal gravity as soon as the slope of the appropriate curve in Fig. 1 exceeds 2/3.

Astrophysical context

For this instability to be cosmologically interesting, A_0 must be > 1 and at the same time fairly large scales must be optically thin. We now investigate the range of astrophysical parameters where this may occur.

It is useful to write the luminosity density in units of the Eddington limit¹⁵:

$$l_E \equiv \rho c^2 / t_{\text{Sal}} \quad (9)$$

where

$$t_{\text{Sal}} = c\sigma_T / 4\pi Gm' \approx 4 \times 10^8 \text{ yr} \quad (10)$$

is the Salpeter time, $\sigma_T \approx 6 \times 10^{-25} \text{ cm}^2$ is the Thomson cross-section, and m' is the mean mass of cosmic atoms. Once again ignoring curvature terms in the expansion law, $H^2 = 8\pi G\rho/3$, we obtain

$$A_0 = \frac{t^2 l \sigma}{mc} = \frac{2}{3} \frac{l}{l_E} \frac{\sigma}{\sigma_T} \frac{m'}{m} \quad (11)$$

If the radiation sources are Eddington-limited, it is apparent that the effective cross-section per gas atom $\sigma m'/m$ must exceed the Thompson value in order for rapid growth to occur.

This requirement is found to be generously satisfied in the interstellar medium (ISM) of our own galaxy. Spitzer (ref. 5, p. 162) finds empirically that the sky coverage per gas atom is

$$\frac{\langle n_{\text{dust}} \sigma_{\text{dust}} \rangle}{\langle n_{\text{H}} \rangle} \approx 10^{-21} \text{ cm}^2 \quad (12)$$

based on measured selective extinction at 1,000 Å. In Spitzer's model, the corresponding dust-to-gas mass ratio is $X_d = 6 \times 10^{-3}$. In what follows we therefore adopt $\sigma m'/m \equiv f_{\text{ISM}} 10^{-21} \text{ cm}^2$. Because $\sigma \propto \lambda^{-1}$ for wavelengths larger than the typical grain size, the value of f_{ISM} is sensitive to the mass in dust grains but not to their size distribution, provided they are small compared with the characteristic wavelength of the radiation. f_{ISM} is also sensitive to the value of this wavelength. A similar opacity per mass would be expected for large molecules^{11-13,18}.

In our discussion of mock gravity, we focus exclusively on grain opacity. Although atomic continuum cross-sections in the ultraviolet are large enough to be interesting ($f_{\text{ISM}} \approx 6000$ for Lyman continuum absorption), atoms are quickly ionized in ultraviolet radiation fields strong enough to produce instability unless the gas is concentrated in small, very dense lumps. Line opacity is ineffective both because line widths are less than the systematic Hubble velocity across the structures that interest us, and because only a small fraction of the radiation participates in the instability. Dust grains or large molecules absorb ultraviolet continuum radiation efficiently but are large enough to survive the absorption of an ultraviolet photon. Grains and molecules tend to survive more easily at earlier epochs than they do now, because the dominant destructive process—sputtering by collisions with energetic ions—is suppressed. Gas at redshift $z > 7$ is cooled by Compton interaction of electrons with the microwave background, and does not reach the high temperatures needed for sputtering⁵.

Formation of structures

If the effective opacity is too high, scales of interest will be optically thick and structure will not grow. Let us require that the instability operates up to a fiducial wavelength $\Lambda_c = 32\Lambda_{32} h^{-1} \text{ Mpc}$. A value of $\Lambda_{32} \approx 1$ seems necessary to produce galaxy density fluctuations of order unity in spheres of radius $8h^{-1} \text{ Mpc}$, as inferred from the observed galaxy autocorrelation function. The optical depth parameter across this characteristic scale is

$$p_c = 2\pi / \Lambda_c n\sigma = 6.5 f_{\text{ISM}}^{-1} \Omega_g^{-1} h^{-1} (1+z)^{-2} \Lambda_{32}^{-1} \quad (13)$$

where we have set the gas density to $n_{\text{H}} = 10^{-5} \Omega_g h^2 (1+z)^3 \text{ cm}^{-3}$ and the current value of $H = 100 h \text{ km s}^{-1} \text{ Mpc}^{-1}$. For the instability to operate efficiently, p_c must exceed unity. Thus for significant growth across Λ_c by $z=20$, we require $\Omega_g < 0.016 f_{\text{ISM}}^{-1} h^{-1} \Lambda_{32}^{-1}$.

Although individual sources might be expected to radiate at the Eddington limit, the mean luminosity density at $t > \epsilon t_{\text{Sal}}$ is likely to be limited by the total efficiency, ϵ , with which rest mass is converted into radiation (this constraint could be avoided, in principle, by having an intense burst of radiation). For a population of sources which forms at $t=0$ and burns uniformly until time t_b ,

$$l/l_E = \epsilon (t_{\text{Sal}}/t_b) = 6 \times 10^{-4} \epsilon_{-2} (\Omega h^2)^{1/2} (1+z_b)^{3/2} (\rho_s/\rho) \quad (14)$$

where ρ_s is the density of sources and z_b is the redshift corresponding to t_b , ρ is the total density, and $\epsilon_{-2} = \epsilon/10^{-2}$ for the sources. For such a population of sources, the optically thin growth parameter is

$$A_0 = 0.9 \epsilon_{-2} (\Omega h^2)^{1/2} (\rho_s/\rho) (1+z_b)^{3/2} f_{\text{ISM}} \quad (15)$$

To obtain rapid growth, structure formation and burn-out must occur at $z_b > 10$. We can eliminate the explicit dependence on the opacity parameter f_{ISM} by requiring $p_c \geq 1$. Then

$$A_0 \leq 6 \epsilon_{-2} \Omega^{1/2} \Omega_g^{-1} (\rho_s/\rho) (1+z_b)^{-1/2} \Lambda_{32}^{-1} \quad (16)$$

where the density parameters Ω and Ω_g refer to the present. This bound is saturated if we identify Λ_c as the wavelength for which $t_c = t_b$. Equation (16) shows that plausible situations exist in which rapid fluctuation growth at $z > 10$ would occur on all scales up to those of galaxy clusters.

What is the efficiency of converting luminous energy to binding energy? Let u_k denote the Fourier coefficient of peculiar

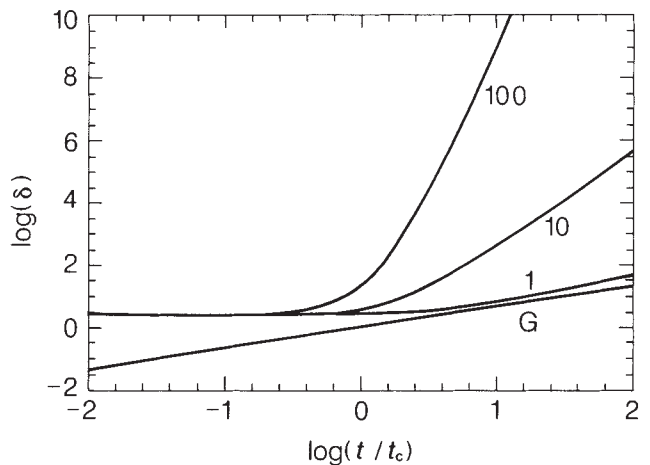


Fig. 1 Growth of perturbations due to mock gravity in the linear approximation. Density perturbation amplitude in arbitrary units is plotted as a function of time, which is expressed in terms of the time t_c when a scale becomes optically thin. The models shown here assume constant opacity and luminosity per mass, and are labelled by the value of their growth parameter A_0 . The curve labelled G represents growth by ordinary gravitational instability.

velocity in the gas. Neglecting the expansion, equation (4) gives us the typical velocity attained in an expansion time:

$$v_{\text{gas}} \approx |u_k/H| \approx |F_k/mH| = \frac{l\sigma\delta_k}{kmcH} [1 - p^{-1} \tan^{-1} p] \quad (17)$$

From equations (9) and (14), the luminosity density at t_b is given by

$$l = \varepsilon \rho_s c^2 / t_b \quad (18)$$

and thus the gas velocity is

$$v_{\text{gas}} \approx 3/2 \varepsilon c \delta_k (\rho_s/\rho) p^{-1} [1 - p^{-1} \tan^{-1} p] \quad (19)$$

Hence, radiation energy is converted to gas motion with maximum efficiency if the scale with $p=1$ goes nonlinear; in this case $v_{\text{gas}} \approx \varepsilon c (\rho_s/\rho_g)$. This corresponds roughly to all the momentum of unidirectional radiation $\varepsilon \rho_s/c$ being imparted to the gas; if $\delta_k=1$ and $p=1$, the radiation field indeed becomes highly anisotropic (see equation (2)). It is interesting that this degree of coherent structuring of the radiation field occurs spontaneously.

If the evolution after t_b is purely gravitational, the binding energy density is $\sim v_{\text{gas}}^2 \rho_{\text{gas}}$. While the maximum mass scale of structure is determined by the opacity (f_{ISM}), the specific binding energy is therefore determined by the radiative efficiency (ε). It is encouraging that we can obtain reasonable results for both simultaneously.

Even during the nonlinear evolution of the instability, radiation pressure acts on optically thin systems exactly like ordinary gravity, but with an effective gravitational constant $3A_0/2\Omega_g$ times larger². As a result, we expect the gas and dust to be swept quickly into a hierarchy of structures. For optically thick systems, the details of the nonlinear evolution driven by mock gravity are complex. Once a system is optically thick, it inhibits the action of mock gravity on both larger and smaller scales. As a result, the growth of structure is coupled on widely disparate scales and is very difficult to treat. Here we discuss two aspects of the optically thin hierarchy: the characteristic masses that emerge and the specific angular momentum generated by mock-tidal torquing.

A characteristic mass scale for nonlinear evolution is defined by the requirements that a contracting clump should become optically thick at the same time as the gas density within it comes to dominate the total background density. Mock gravity then begins to switch off just as normal gravitational effects become nonlinear. This mass M_1 satisfies

$$M_1/\pi r_1^2 = m/\sigma; \quad 3M_1/4\pi r_1^3 = \rho_0(1+z)^3 \quad (20)$$

and hence

$$M_1 \approx 2 \times 10^{16} f_{\text{ISM}}^{-3} (\Omega h^2)^{-2} (1+z)^{-6} M_\odot \quad (22)$$

$$r_1 \approx 30 f_{\text{ISM}}^{-1} (\Omega h^2)^{-1} (1+z)^{-3} \text{ Mpc}$$

For the plausible parameters $\Omega h^2 = 0.25$, $z = 20$, $f_{\text{ISM}} = 0.5$, these give $M_1 = 4 \times 10^{16} M_\odot$, $r_1 = 30$ kpc. Thus this mass scale might be identified with individual galaxies.

A consequence of the rapid growth rate is that the specific angular momentum acquired by gas clumps as they form is typically a factor $\frac{3}{2}(\delta/\delta) = \frac{3}{2}\sqrt{A}$ larger than that which similar clumps would acquire if they turned around at the same epoch under the action of normal gravity alone. As a result, the rotational kinetic energy of a gas clump becomes comparable to its normal gravitational binding energy when it is $(9A/4)$ times larger than in the familiar normal-gravity case^{19,20}. Thus a system which has virialized at size r from maximum expansion size r_m has typical dimensionless spin parameter $\lambda = |E|^{1/2} J/GM^{5/2} \approx 0.06 (9A\Omega r_m/8\Omega_g r)^{1/2}$ if it is gas-dominated. It appears that with little or no collapse, mock gravity can make rotationally supported systems with the parameters of spiral galaxies at a redshift of ~ 20 .

The maximum gas mass over which the instability can operate

effectively is larger than M_1 (equation (22)) by a factor Ω_g^{-2} :

$$M_{\text{max}} = \frac{4\pi}{3} \bar{\rho}_g \left(\frac{m^3}{\bar{\rho}_g \sigma} \right) \\ = 4 \times 10^{16} \Omega_g^{-2} (1+z)^{-6} (\Omega h^2)^{-2} f_{\text{ISM}}^{-3} M_\odot \quad (23)$$

This mass is naturally identified with galaxy clusters. For the parameters suggested above together with $\Omega_g = 0.02$, $M_{\text{max}} = 2 \times 10^{14} M_\odot$, which is comparable to the total mass of gas and galaxies seen in the largest nonlinear systems known. If the instability is able to drive perturbations on this scale mildly nonlinear ($\delta \rho_{\text{gas}}/\rho_{\text{gas}} \sim 5$) at $z = 20$, then systems of total mass $\sim 10^{15} M_\odot$ will collapse as a result of normal gravitational instability at $z \approx 1$ and will contain ~ 10 times as much 'dark' matter as gas and visible stars.

We have made several arbitrary assumptions about the time evolution of luminosity and opacity. If l remained high while the gas density were gradually depleted, the remaining gas would be swept up into ever larger structures, limited by the current optical depth in the remaining gas. Thus, a small fraction of galaxies—those which formed last—might appear in nonlinear structures with a very large coherence length. It might be possible to explain in this way the observed superclustering of Abell clusters, which appears to be coherent over $> 50 h^{-1} \text{ Mpc}$. As we have demonstrated, the gravitational binding of these very large-scale structures is not expected to be large. Large-scale peculiar velocities in the gas of order HR occur while the structure is forming but decay thereafter like $a(t)^{-1}$. The coherence length should scale roughly as the inverse fraction of gas remaining.

Astrophysical implications

The notion that gas clustered first by non-gravitational processes and that dark matter followed its gravitational influence leads to several predictions about galaxy-mass segregation. First, the structure of haloes might be expected to correspond to spherical infall models^{21,22}. Second, the mass-to-light ratio of many-galaxy systems is expected to be simply related to their crossing times. When mock gravity switches off, a highly clumped gas and galaxy distribution will remain superposed on a relatively uniform background of dark matter. A region where the gas density is $(j+1)\bar{\rho}_g$ at this time will later collapse to a system with virialized gas density, $\rho_{\text{vir}} \sim (j+1)\bar{\rho}_g (j\Omega_g)^3$, and with a gas-to-total mass ratio, $\rho_{\text{vir}}/\rho_{\text{tot}} \sim (j+1)\Omega_g$. The crossing time, t_c , of such a system is proportional to $\rho_{\text{vir}}^{-1/2}$, and so objects that form at late times should obey the scaling relation $M/L \propto t_c^{2/3}$. If, in addition, we assume that the hierarchical clustering induced by mock gravity leads to a present distribution obeying $\rho_{\text{vir}} \propto r^{-2} \propto M_g^{-2}$ as inferred from observed galaxy correlations, we can derive the scaling relations, $M/L \propto L^{1/2}$ and $L \propto V^{1/4}$, where V is the characteristic virial velocity. These relations are in reasonable agreement with observations of galaxy systems.

The stellar population required in our model cannot have the same mass function as that in our Galaxy; normal stars with a large ultraviolet flux and lifetimes $< 10^8$ yr eject too large a fraction of their mass in metals to produce the needed light without over-enriching the remaining gas. One promising type of radiation source for our purposes would be a 'very massive object'^{8,9} ($M \geq 200 M_\odot$), which sends nearly all its metals into a black hole and blows off an envelope of hydrogen and helium. Radiative efficiencies for such objects typically^{8,9} exceed 10^{-3} . The effects of the explosions from such objects could possibly dominate over their mock-gravity effects if they formed at sufficiently low redshift ($z < 6$). Accreting black holes could also match our luminosity requirements without creating an enrichment problem, and could reach even higher efficiencies.

On the other hand, an appreciable metal abundance ($\leq 10^{-3}$, depending on luminosity), is necessary in the protogalactic gas to provide sufficient dust opacity for the instability to operate. In this model, it is possible that a significant portion of present galactic dust originated at high redshift; this is a desirable feature

as present dust formation rates seem inadequate²³. The high metallicity of the intergalactic gas in galaxy clusters may result from sputtering of pre-galactic grains once Compton cooling has turned off at $z \approx 7$. Observed objects with low metallicity are not a serious embarrassment because mock gravity segregates gas according to its dust content. Initial chemical inhomogeneities thus tend to get amplified, highly enriched regions being brought together while metal-poor gas is left behind. This segregation can occur even though dust and gas may be locally well coupled, because enriched clouds are compressed by radiation pressure and can pass through the unenriched gas with little interaction: some such segregation would be expected even on scales as large as M_{max} , provided the enriched clouds are compressed to $\tau \geq 1$. Thus, the existence of metal-poor systems such as IIZw40 may not be in conflict with our model. We would, however expect such systems to be less strongly clustered than normal galaxies. There are physical reasons why globular clusters may form preferentially from low-abundance clouds in protogalaxies²⁴, and there is some observational support for just such selective cluster formation²⁵. The low metal abundance of clusters in the galactic halo may, therefore, be compatible with the substantial pre-galactic metal formation our model requires. Such relations between metallicity and structure may also explain why Lyman- α absorption systems in quasars are less strongly clustered than metal-line systems, and indeed why there should be a correlation between column density and metallicity.

Perhaps the cleanest prediction of our model—because it is a direct by-product of the fundamental mechanism—is the intensity and anisotropy of the re-radiated emission from the grains. This is expected to produce a cosmic far-infrared background peaked at $200 \mu\text{m} \leq \lambda \leq 600 \mu\text{m}$ (ref. 10). The total present energy density parameter in radiation from sources which burned from $z = \infty$ to $z = z_b$ is

$$\Omega_R = \frac{3}{5}(\rho_s/\rho)10^{-2}\epsilon_{-2}(1+z_b)^{-1}\Omega \quad (24)$$

Using equation (16) and requiring $A_0 > 6$ so that the instability can operate, we find

$$\Omega_R > 0.6 \times 10^{-2} \Omega^{1/2} \Omega_g \Lambda_{32} (1+z_b)^{-1/2} \quad (25)$$

which should be compared with the total energy density of the cosmic microwave background, $\Omega_R = 2.4 \times 10^{-5} h^{-2}$. Even for $z > 10$ and $\Omega_g \approx 0.01$, the two fluxes are comparable. Such a strong far-infrared background should be easily detectable by the Cosmic Background Explorer Satellite (COBE).

The anisotropy in this background may be estimated as follows¹⁰. Suppose that the re-radiated infrared luminosity density has variance $(\delta I/I)_{\text{IR}} \approx 1$ on the co-moving scale $\Lambda \approx 32 h^{-1} \Lambda_{32}$ Mpc of observed clustering. Roughly $N \approx 100 \Lambda_{32}^{-1} h^{-1} (1+z_b)^{-1/2}$ such regions contribute to the dust emission integral in any direction, so the r.m.s. variation in observed brightness will be

$$\frac{\delta I}{I} \approx N^{-1/2} \approx 0.1 \Lambda_{32} h^{1/2} (1+z_b)^{1/4} (\theta/\theta_c)^{-1} \quad (26)$$

for angular scales θ larger than that subtended by Λ_c .

$$\theta_c \approx 16 \Lambda_{32} \Omega \text{ arc min} \quad (27)$$

Anisotropy with these characteristics should be detectable by the Space Infrared Telescope Facility (SIRTF). The techniques for calculating small-scale anisotropy, and the observational issues, are discussed in ref. 10.

These fluctuations in the dust radiation can generate further inhomogeneities in the matter distribution as a result of coupling by Thomson drag¹⁷. This effect is negligible for the parameters we prefer here, but it could be significant on large scales if dust and radiation sources were present at $z \geq 200$ in sufficient quantities to generate a small-scale mock gravitational instability. If the mock-gravity instability occurs at high enough redshift or gas density, the Thomson optical depth may exceed unity, suppressing anisotropy on angular scales less than a few degrees¹⁷. This does not occur, however, for parameters which produce large-scale structure.

Discussion

The model we have presented is radically different from conventional models for galaxy formation. In view of this, it is surprising that many observed facts fall into place quite 'naturally' and that no fatal flaw has appeared. Apparently, the model deserves serious consideration. Unfortunately, however, there is considerable difficulty in sharpening many of its predictions. The nonlinear evolution of the structure in mock gravity is particularly difficult to analyse rigorously, and even numerical simulations are difficult because the force between two gas elements in optically thick systems depends on the global configuration of the system. It may be possible to construct an apparatus in which mock gravity between many particles could be observed directly. This would offer the advantage of representing most aspects of the phenomena in question with a very large number of particles and with high spatial resolution and dynamic range. The acceleration of a grain in an evacuated chamber with $\sigma = 10^{-8} r_\mu^2 \text{ cm}^2$ and $m = 10^{-12} \rho_g r_\mu^3 \mu\text{g}$ due to a beam of radiation with an energy flux of $100 F_{100} \text{ W cm}^{-2}$ is

$$a = 300 \text{ cm s}^{-2} F_{100} r_\mu^{-1} \rho_g^{-1} \quad (28)$$

Thus, it may be possible to build an apparatus in which the mock gravitational acceleration exceeds normal terrestrial gravity ($g = 981 \text{ cm s}^{-2}$), or the mock-gravity instability proceeds so rapidly that the entire apparatus can be dropped briefly to eliminate normal gravity during the experiment. It would be amusing to build an analog computer in the laboratory that might allow us to study the formation of galaxies and galaxy clusters in the real Universe.

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Voltage-sensitive dyes reveal a modular organization in monkey striate cortex

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Voltage-sensitive dyes allow neuronal activity to be studied by non-invasive optical techniques. They provide an attractive means of investigating striate cortex, where important response properties are organized in two dimensions. In the present study, patterns of ocular dominance and orientation selectivity were obtained repeatedly from the same patch of cortex using the dye merocyanine oxazolone, together with current image-processing techniques. The patterns observed agree with most established features of monkey striate cortex and suggest a new unit of cortical organization; one that is modular in structure and which appears to link the organization of orientation selectivity with that of ocular dominance.

NEURONES in the striate cortex perform several important transformations using visual information¹⁻⁸. They combine inputs from both eyes so that retinal images appear fused, yet they retain information on ocular disparity, so that objects may be viewed in relief. They also respond selectively to edges at certain orientations in visual space. The two response properties most related to depth and edge perception, ocularity and orientation selectivity, are organized systematically across the cortical surface.

The anatomical basis for ocular interaction in the cortex is better understood than that of orientation selectivity because it originates from patterns of terminating geniculate afferents and there are methods to visualize these afferents. The geniculate axon terminals establish a repeating sequence of right-eye/left-eye bands in lamina 4C (refs 5, 8, 9) which, when viewed from the cortical surface, display an average width of 0.5 mm and run vertically into the boundary of area 17 (striate cortex). As information flows radially out of lamina 4C (ref. 10), the monocular responses that these bands establish carry over into, and dominate, the responses of cells lying above and below, creating a system of slab-like columns that extend from pia to white matter.

Orientation selectivity, like ocular dominance, seems to be organized radially (vertically between pia and white matter)^{1,2,6,8}, but its precise anatomical substrate is unknown, and its organization has thus proved more difficult to define¹¹. Using long tangential electrode penetrations, Hubel and Wiesel found that incremental steps through the cortex are accompanied by incremental shifts in preferred orientation⁶. This led them to suggest that orientation selectivity, like ocular dominance, might be organized as a system of parallel slabs, and experiments with 2-deoxyglucose (2DG), a metabolic marker that labels active neurones, seemed to confirm this notion¹². But these experiments suffer from the fact that 2DG can be used only once (the animal must be killed to visualize the result), and this makes it difficult to determine how the same piece of tissue might respond to a different stimulus (a different orientation, for example). The presence of cytochrome oxidase blobs (identified on the basis of their rich cytochrome oxidase content), at regular intervals in the superficial layers, complicates the issue further since cells within these blobs lack selectivity for orientation and label strongly with 2DG for any orientation that is used. On account of all these difficulties, the evidence for an overall slab-like organization has never seemed quite as compelling for orientation selectivity as it has for ocular dominance¹³⁻¹⁵.

A technique that might perhaps allow repeated mapping of cortical activity patterns was reported by Cohen and co-workers, who showed that certain dye molecules function as optical transducers of membrane potential¹⁶⁻¹⁹. Squid axons stained

with merocyanine 540 were found to emit a fluorescence signal that closely followed action potentials measured with an intracellular electrode¹⁶. The mechanism(s) responsible for these optical signals are not understood completely, but are thought to entail a voltage-dependent transition between monomer and dimer states of membrane-bound dye molecules¹⁹. Rotations of dye molecules within the membrane²⁰ or rotations of dipole moments during monomer to dimer transitions may also be involved^{19,21}.

While voltage-sensitive dyes may seem a complicated way to monitor membrane potentials, they offer great promise in the study of two-dimensional phenomena because surfaces are well suited to optical inspection. Although initially the small amplitude of voltage-dependent optical signals and the phototoxicity of the dyes restricted widespread application of the method^{16,18,22}, many of these problems have now been overcome¹⁹⁻²² and it is possible to obtain optical signals from many different neural preparations, including mammalian cortex²³.

We report here results achieved with one such potentiometric dye, merocyanine oxazolone (NK2367)²⁴, on the organization of the monkey striate cortex. Our approach differed from that of previous investigators in that we used video imaging technology instead of photodiodes^{23,25,26} to monitor activity. This sacrificed temporal resolution (which was not needed in the present study as ocular dominance and orientation are stable over time), but allowed us to achieve greater spatial resolution than previously possible. As a result, we observed patterns of activity not visualized before, including direct images of ocular dominance bands in living animals and the first detailed maps of orientation selectivity covering large areas of cortex. Analysis of these patterns revealed that orientation changes continuously within discrete domains, or modules, and that these modules overlie ocular dominance borders in such a way that ocular dominance varies continuously within them.

Monkeys

We used macaque monkeys for these studies because this species has contributed most to our understanding of the striate cortex. Macaque monkeys offer the additional advantage that large areas of striate cortex (corresponding to central and parafoveal parts of the visual field) lie in the relatively flat and exposed operculum directly under the cranium at the back of the head. Three animals that served as subjects in unrelated experiments were used to test and develop the chamber and optical apparatus. However, the results reported here were achieved with only two animals (one *Macaca nemestrina* and one *Macaca fascicularis*). Both were implanted with chronic chambers that allowed us to record from single patches of cortex on successive occasions over a period of eight weeks.

Monkeys were anaesthetized and paralysed according to established procedures^{3,27}. Both eyes were refracted and fitted with contact lenses and 3-mm artificial pupils. Preliminary recordings with microelectrodes established visual field locations for the exposed cortex and allowed us to align each eye separately with the centre of a computer-driven 19-inch monitor.

Optical apparatus

Figure 1 shows the apparatus used. Light from a tungsten halogen lamp passes through a collimator, interference filter and diaphragm before being reflected onto the cortical surface. An objective lens (0.1–0.3 numerical aperture) collects the reflected light, passes it to a projection lens to focus an image of the cortex either onto a set of four photodiodes or to a Newvicon television camera (COHU Inc., Model 5300, San Diego, California). Outputs from the photodiodes were fed into conventional current-to-voltage converters, amplified and sampled by an A/D converter. The video signal was fed into an image processor (Imaging Technology Inc., Woburn, Massachusetts) which consisted of a digitizer with a flash 8-bit A/D converter (AP-512), four frame buffers (FB-512-Q) and an arithmetic unit processor (ALU-512-Q). Video images taken under different conditions of visual stimulation were accumulated in separate frame buffers for subsequent manipulation by the processor. Because of the small size of the expected signals (1 part in 1,000 or less) and the limited dynamic range of the A/D converter (256 grey levels), additional circuitry was required: (1) to flatten the signal (to shade the video image so that all parts had roughly equivalent values); (2) to boost the small signal gain (which contains most of the voltage-dependent information); and (3) to eliminate d.c. bias (reduce the apparent white level). This additional circuitry put radiant changes $>0.01\%$ within the range of the flash A/D converter; it also introduced and amplified noise which was removed through averaging. Tests with the calibrated emissions of a light-emitting diode indicate that this system detects radiant changes greater than 0.01% if they persist long enough (of the order of seconds) to be averaged. Improvements in the signal/noise ratio at the front end (for example, by cooling the image detector) should shorten the duration of required averaging.

Cortex staining

The dura was removed and the exposed cortex stained by circulating a 0.1% solution of NK2367 (Nippon, Kankoh-Shikiso, Kenkyusho, Ltd., Okayama, Japan) in balanced salt solution over the surface for 0.5–1 h. This solution was then washed out and the inlet and outlet tubes sealed to minimize cortical pulsations. Sections from freshly stained monkey cortex indicate that this procedure allows NK2367 to penetrate ~ 0.5 mm into the cortex. Because the observed signals strengthen steadily during the first 0.5–1 h after staining, it seems that dye in the more superficial layers (probably in the pia) must be bleached before optical signals from the deeper tissue become visible.

Observations

A set of four photodiodes were placed in the image plane of our optical apparatus (Fig. 1) to monitor optical signals simultaneously from separate patches of cortex ($100\text{ }\mu\text{m}$ wide, separated by at least $400\text{ }\mu\text{m}$). This arrangement allowed us to screen several membrane-permeable cyanine and impermeant merocyanine dyes known to exhibit voltage-dependent optical responses. Several dyes yielded signals, but those achieved with NK2367 stood out to such an extent that we concentrated all subsequent efforts on experiments with this dye.

Figure 1b shows the signal from one photodiode averaged over 10 presentations of vertical and horizontal gratings. Movement artefacts associated with heartbeat and respiration were

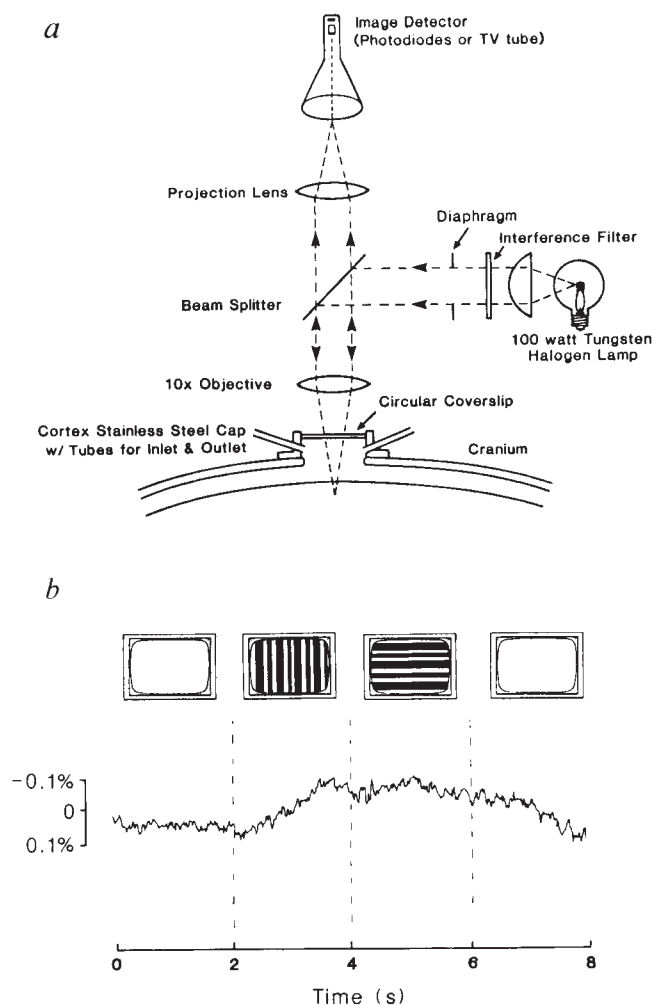


Fig. 1 *a*, Experimental arrangement for monitoring voltage-sensitive dyes in monkey striate cortex. A hole (25 mm diameter) was cut in the cranium, a stainless-steel ring cemented to the skull and the dura removed. The ring had stainless-steel inlet and outlet tubes to bathe the cortical surface with dye solution (0.1% in balanced salt solution). A second ring containing a round glass coverslip was screwed into the first ring to form a water-tight compartment. The position of the glass could be adjusted to dampen cortical pulsations and provide an optically smooth window for viewing the cortex. A condenser lens collimated light from a 100-W tungsten halogen lamp and directed it through an interference filter (720 ± 25 nm, Omega Optical, Brattleboro, Vermont), an aperture, then to the reflective surface of a beam splitter (a 50% half-silvered mirror). Reflected light passed through an objective lens and glass coverslip onto the cortex. Reflected and scattered light from the cortex passed through the coverslip, to the objective lens, through a projection lens, to the image detector (photodiodes or the TV camera). *b*, Averaged response to 10 successive presentations of vertical and horizontal gratings as seen by one photodiode that surveyed a $100\text{-}\mu\text{m}^2$ patch of cortex. At the onset of a drifting vertical grating, there is a sudden increase in absorption for this part of cortex. A small glitch appears where the grating changes from vertical to horizontal, and the trace returns to baseline when the horizontal grating fades back again into a blank screen. Sequential presentations were synchronized to both the electrocardiogram and the respiration so that movement artefacts produced by these events could be subtracted, as previously described by Orbach *et al.*²³.

eliminated from this trace by synchronizing each presentation to the animal's electrocardiogram and respiration²³. In this trace, an increase in absorption (at $700\text{--}740$ nm) corresponds to the appearance of a drifting vertical grating. A small glitch appears approximately at the time the vertical grating is replaced by a horizontal one, and the signal begins to decay back to baseline

Fig. 2 *a*, Cortical blood vessels viewed under white light. Blood vessels appeared out of focus because the optical system was focused 300 μm below the pial surfaces. This patch of cortex was located just behind the lunate sulcus in the right hemisphere. Posterior, anterior, medial and lateral correspond to the top, bottom, right and left of the image frame, respectively. The border between areas 17 and 18 lies parallel to and just below the lower border of this frame. The height and width of this image corresponds to a $4 \times 5\text{-mm}$ patch of cortex. *b*, The familiar pattern of ocular dominance bands is produced by subtracting images accumulated during visual stimulation of the contralateral eye from images accumulated during stimulation of the ipsilateral eye (all orientations). This produces an intensity difference of $\sim 0.2\%$ (amplified electronically) between the dark and light bands. The bands have an average width of 0.5 mm, run vertically into the area 17/18 border, and correspond to the rows of cytochrome oxidase 'blobs' which appear in *d*. The spots in *b* illustrate the location of 16 electrode penetrations conducted in this region of cortex before and after measurements with voltage-sensitive dyes. Small black dots indicate sites that yielded binocular responses, open squares indicate sites that were dominated by the contralateral eye, and closed squares indicate sites dominated by the ipsilateral eye. In agreement with the photodiode results, the correspondence between the dark bands and closed squares indicated that stained cortical tissue darkens with increased electrical activity (see text). *c*, The result of subtracting two time-averaged images while the monkey viewed a blank screen. The same algorithm was used as in *b*. *d*, At the end of the experiment, the same piece of cortex was sectioned tangentially and stained for cytochrome oxidase³⁶. The section image shown here was aligned and expanded (necessary because of tissue shrinkage) according to the positions of blood vessels so that they laid in register with the images in *a*–*c*. Note that during examination of the histological preparations, no evidence was found of tissue damage attributable to repeated applications of the dye over more than eight weeks.

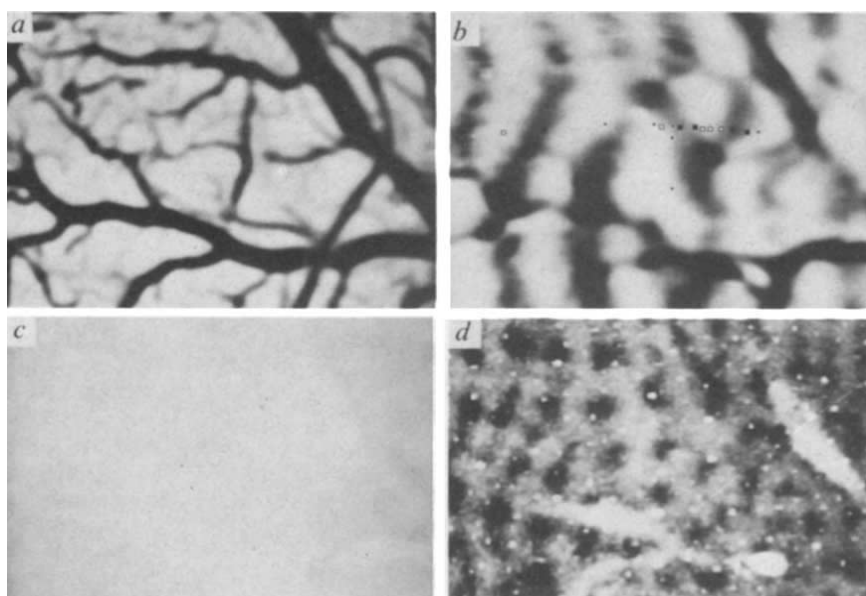
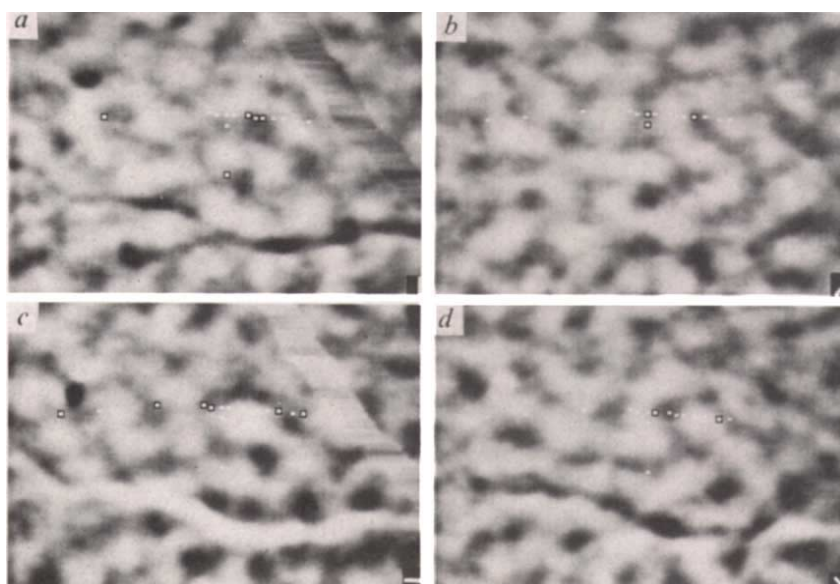


Fig. 3 Iso-orientation activity patterns produced by four separate orientations—vertical, right oblique, horizontal and left oblique. Each image was compiled separately and related to a reference image generated by the orthogonal orientation. This subtraction was necessary to minimize the effects of activity-dependent hyperaemia²⁸. For example, images of cortex taken during horizontal presentations were subtracted from images taken during vertical presentations to produce the pattern in *a*. The observation that vertical and horizontal patterns shown in *a* and *c* are complementary merely underscores the consistency of the result. White dots indicate the positions of electrode recording sites. Those outlined in black denote locations where sampled cells preferred orientations within 22.5° of that presented. These correlate with the darker parts of each image and never overlap with the lighter ones. Major blood vessels, which sometimes produce shadows caused by uncontrollable dilation during image acquisition, have been blocked out to prevent unwanted influences on adjacent regions of the same image during subsequent normalization and image processing.



when the horizontal grating disappears. The occurrence of similar responses for horizontal and vertical gratings (increased absorbance in both cases) is probably due to the location and size of the cortical patch surveyed.

The large sizes, stability and long durations of these signals suggested that they might be monitored with higher spatial resolution using a TV camera. Stable signals over a long period made it possible to average many frames during each visual presentation; typically, 1,800 frames (30 frames per s) were summed during a set of 10 presentations. This led to a profound improvement in the signal to noise ratio; and further improvements were achieved through lateral averaging—that is, by dividing the image into squares 4 pixels high and 4 pixels wide, and

averaging all the pixel values within each square. As noted above, the paradigm detects radiant changes greater than 0.01%.

Ocular dominance

Figure 2*a* shows a $4 \times 5\text{-mm}$ patch of striate cortex as it appeared under white light. Blood vessels appear out of focus because the focal plane lies 300 μm beneath the pial surface. The lunate sulcus, as well as the accompanying border between areas 17 (striate cortex) and 18, lie parallel to and just below the lower boundary of the image. Blood vessels do not present a problem because they become virtually transparent to light at 720 nm (the wavelength at which we monitor the response of the dye), and disappear altogether (except for a couple of the largest

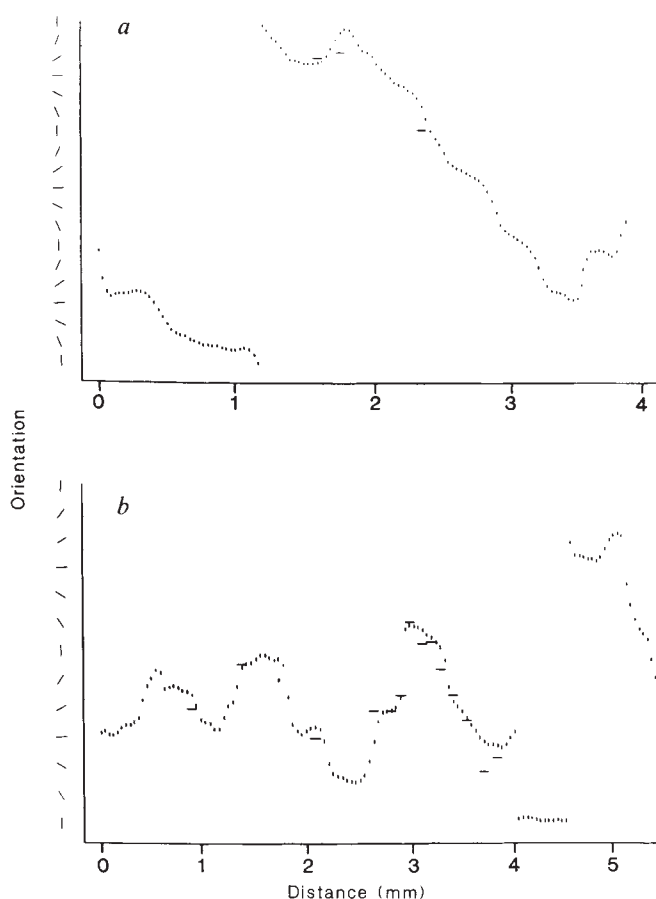


Fig. 4 Orientation values as they would appear along a single, tangential electrode penetration. This is done twice. *a*, Orientation values along a vertical scan line running through the three vertically aligned sites in the centre of Fig. 5*a*. *b*, Orientation values along a single horizontal scan line lying along the 14 horizontally aligned sites in Fig. 5*a*. Short horizontal lines indicate the actual orientation values that we determined (by averaging orientation preferences determined at three different depths) for each of the sites.

vessels) when one cortical image is subtracted from another (Fig. 2*c*). Because of the strong haemoglobin absorption at 430–610 nm it is unlikely that such controls could be obtained at wavelengths below 620 nm.

Figure 2*b* depicts the characteristic pattern of ocular dominance bands produced by subtracting cortical images accumulated during visual stimulation of the contralateral eye from images accumulated during stimulation of the ipsilateral eye. Because cortical tissue darkens with activity once it has been stained with NK2367, the dark bands in Fig. 2*b* correspond to ipsilateral dominance. Comparison of Fig. 2*a* and *b* shows that these bands have little in common with blood vessels. However, there is a tendency for the largest vessels to produce shadows (either light or dark), largely through uncontrollable changes in vascular perfusion during neuronal activity²⁸.

Besides resembling ocular dominance bands visualized with other techniques^{5,8,9}, the bands in Fig. 2*b* display several characteristic features: they have the same width as those observed with 2DG, they run vertically into the border of area 17, and every light and dark stripe straddles a row of cytochrome oxidase blobs, as would be expected¹³. The latter correlation is apparent from the aligned image (Fig. 2*d*) of a section from the same patch of cortex which was stained for cytochrome oxidase.

To confirm the identities of these bands independently, direct electrical measurements were made with microelectrode

penetrations at 16 separate locations. The spots in Fig. 2*b* indicate ocular dominance values determined (on the basis of recordings at three separate depths) for each location. The round spots (indicating balanced binocular responses) predominate near the edges of bands while the open squares (contralateral dominance) align with the light bands and the closed squares (ipsilateral dominance) with the dark.

Orientation

Initial attempts to visualize orientation selectivity were made by subtracting images obtained during control conditions (equiluminant grey screen) from images obtained while the monkey viewed a drifting vertical grating. This approach was hampered by the previously mentioned vascular changes induced by neuronal activity²⁸, and by a diffuse, activity-dependent darkening of the cortex which may be the result of generally heightened activity within the stained cortical neuropil.

These problems can be avoided by comparing frames accumulated during stimulation with two orthogonal orientations. Activity remains high for both sets of frames such that perfusion and diffuse changes remain constant, and only events induced by a change in the stimulus orientation are detected.

We used this approach to produce the four images in Fig. 3, all of which resemble patterns achieved with 2DG¹². The patterns produced by horizontal and vertical gratings complement one another, as do patterns produced with right and left oblique. Although this finding might appear self-evident given our subtraction protocol, the lack of overlap between the patterns reinforces their stimulus-relatedness and stability, and reduces possible concern about spontaneous fluctuations in neuronal activity. The image in Fig. 3*c*, for example, was obtained half an hour (and six intervening scans) after that in Fig. 3*a*.

Small white squares in Fig. 3 indicate the positions of our 16 electrode penetrations. Those revealing an orientation preference within 22.5° of the test stimulus are outlined in black. It is easy to see that the latter predominate within active (darkened) regions of cortex and avoid inactive (light) regions.

To improve our resolution of orientation selectivity, we obtained 12 separate activity patterns from the same patch of cortex taken at 12 separate orientations (that is, at 15° intervals). These allowed us to compare values at each pixel location (for every image) to determine the orientation responsible for the strongest response. Figure 5*a* depicts the colour-coded optimal orientation at each point, and shows that orientation values change continuously in most regions of cortex and that all orientations are represented within fairly small areas (<1 mm²). It also allows the extraction of orientation values along single scan lines so that they may be plotted directly. This is done in Fig. 4*a* and *b*, which shows orientation values along two orthogonal axes that intersect the sites of electrode recordings (white squares in Fig. 3*a*). These plots resemble qualitatively those of Hubel and Wiesel's tangential electrode penetrations⁶, and agree with the results of our own recordings (indicated by short horizontal lines).

Fractures

The colour-coded images in Figs 5*a* and 6*a* highlight the distribution of regions responsive to different orientations in one patch of cortex. This distribution is not one of parallel bands since the image does not have an overall rainbow appearance, but it is possible to find small regions where such an appearance is predominant; these regions are separated from one another by sudden shifts in preferred orientation which break the continuity.

To visualize the pattern of disruption more completely, we applied a gradient operator to the orientation values in Figs 5*a* and 6*a*. This is a simple operator which derives the rate of orientation shift (like a first derivative) in each part of the image. The results appear colour-coded in Figs 5*b* and 6*b*, where the colour sequence orange, yellow, green, blue, red and black

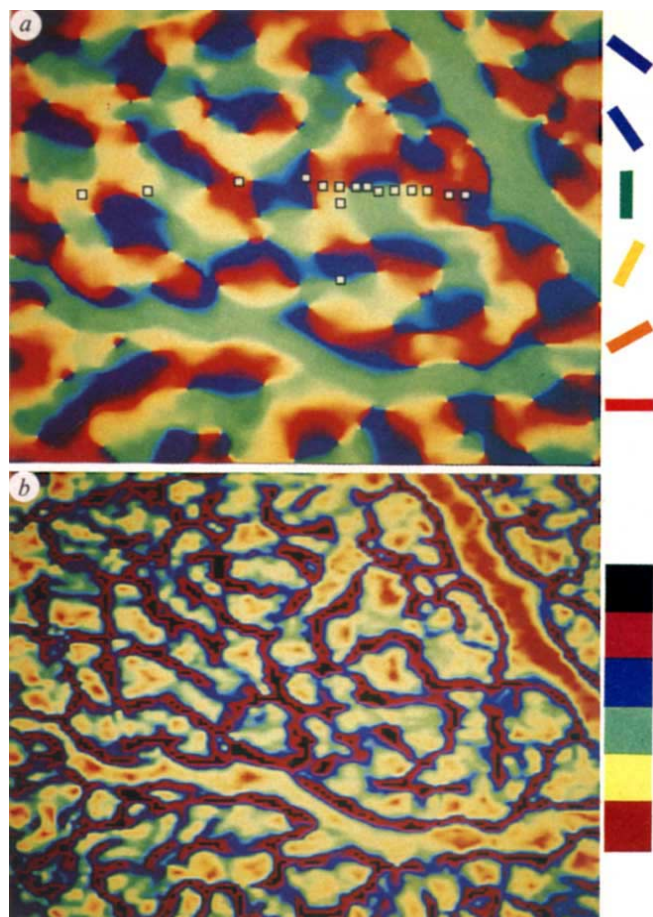
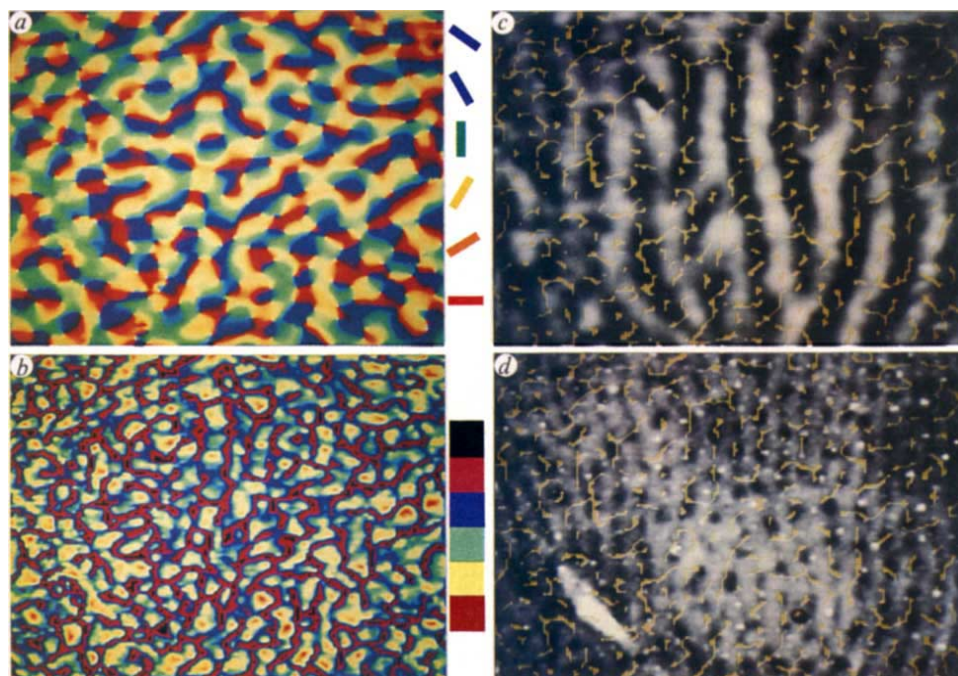


Fig. 5 *a*, Detailed orientation preferences mapped from the same patch of cortex using successive presentations of 12 separate orientations. Like the images in Fig. 3, these patterns were generated by collecting two sets of frames with orthogonal orientations, as the animal viewed bi-directionally moving gratings, and subtracting one from the other. The process was then repeated with different orthogonal pairs. Our computer (LSI 11/73) used the 12 resulting images to analyse orientation selectivity at every point in the image. Every pixel in each image was transformed into a vector; the magnitude of each vector was the pixel value (response intensity), and its angle was twice the orientation of the stimulus grating. This representation ensured that for each pixel, vectors representing equivalent responses at orthogonal orientations would cancel out. The 12 vectorial images were then summed to produce an output image consisting of two parts, one corresponding to vector magnitudes which reflects the degree of orientation selectivity for each part of cortex, and one corresponding to half the vector angle, reflecting orientation preferences. This figure shows colour-coded representations of optimal orientations. Actual orientation values appear graphically in Fig. 4. It is important not to overinterpret areas devoted to any particular orientation as most of the apparent differences derive from nonlinearities in colour processing (particularly with respect to blue). *b*, Gradient of the orientation values presented in *a*, taken so as to visualize the two-dimensional distribution of continuous regions and sudden breaks. The colours orange, yellow, green, blue and red indicate increasingly rapid rates of orientation change; black denotes regions where orientation preference jumps by 45° or more within a distance of $40\ \mu\text{m}$.

Fig. 6 Low-power view of the striate cortex. The horizontal width of this image corresponds to 8 mm on the cortical surface. As in Fig. 5, the area 17/18 border lies parallel to and just below the lower border of this image. *a*, Colour-coded distribution of orientation, similar to that shown in Fig. 5*a*. If orientation selectivity really were organized into parallel slabs, this image should look like a rainbow, or stacks of rainbows. Such colour distributions are present only on a small scale. *b*, Colour-coded representation of the orientation gradient. At this lower power, modules of continuous orientation domains are even more apparent than in Fig. 5*b*. As in Fig. 5*b*, the most abrupt transitions—those exceeding 45° in $40\ \mu\text{m}$ —are indicated in black. *c*, Ocular dominance pattern for the same part of the cortex (obtained by alternate visual stimulation of the two eyes, as in Fig. 2*b*). Fine yellow lines indicate exceptionally radical shifts in orientation preference and correspond approximately to the black regions in *b*. Note the pronounced tendency for these lines to run down the centres of adjacent ocular dominance columns, or to intersect them at right angles. If we lower the fracture threshold to include slightly less radical jumps in preferred orientation, these lines elongate, becoming more modular in appearance, and eventually converge to form a pattern similar to that in *c*. The fracture-bounded modules are situated between the centres of adjacent ocular dominance columns, an opportune place for receiving balanced inputs from both eyes. This arrangement also establishes a continuous shift in ocular dominance across each module. *d*, Cytochrome oxidase-stained section from the same patch of cortex. Even a casual comparison between this image and that produced in *c* shows the close correspondence between cytochrome oxidase blobs and the centres of optically determined ocular dominance columns (both light and dark). As in *c*, yellow lines indicate the positions of fractures. Due to their pronounced tendency to align with the centres of ocular dominance columns, the fractures also overlap with the cytochrome oxidase blobs, an observation perhaps related to Livingstone and Hubel's finding that orientation tuning weakens and sometimes disappears near blobs¹⁵. Maps of orientation tuning (derived from the vector magnitude of the operation that produced the images in 5*a* and 6*a*) reveal a decreased selectivity for orientation in the vicinity of blobs and fractures (not shown).



indicates increasingly rapid changes (with respect to distance) in preferred orientation. It is clear from this representation that the most abrupt changes (indicated by red and black) travel along lines similar to fractures in a crystal, and circumscribe small (0.5–1 mm wide) regions of cortex. Within each region, orientation changes continuously along one axis, in a manner consistent with an organization of parallel slabs. Because these regions occupy most of the cortical surface area, and clearly are circumscribed by fractures, one can think of them as modules. The latter appear highly regular in area, and where larger areas are observed they take the form of elongated, module-wide strips, suggesting that smaller domains with mutually confluent maps have merged.

Fractures and ocular dominance

As both orientation selectivity and ocular dominance repeat their representation within short distances (<1 mm), it seems likely that they are related. One possibility, suggested by Hubel and Wiesel^{6,7}, but which they subsequently disproved¹⁰, is that iso-orientation bands intersect ocular dominance columns at some consistent angle. Our results also reject this notion. But when we compare ocular dominance columns with sudden changes in preferred orientation (that is, with the fractures), we find a marked correlation. As can be seen from the superposition of ocular dominance columns and fracture segments in Fig. 6c, the fracture segments that are aligned with ocular dominance columns run down their centres and avoid the edges. Those that intersect the boundaries of two adjacent columns do so at right angles. Accordingly, disruptions in the gradient of preferred orientation tend to coincide with reversals in the gradient of ocular dominance; and orientation and ocular dominance both vary continuously along single but separate axes within each module.

Comments

Our results demonstrate the feasibility of using voltage-sensitive dyes in conjunction with video imaging techniques to study functional organization in the monkey striate cortex. They reveal for the first time a detailed map of cortical regions responsive to different orientations. We will discuss these two very different findings separately.

Dye technology. One important issue concerns the source of our optical signals. Although we have noticed a general, activity-dependent darkening of unstained cortex, we have not found this phenomenon useful in identifying cortical response properties. We believe that changes in dye absorbance underlie the generation of the patterns we see because: (1) these patterns do not become apparent until after the cortex has been stained; (2) they become weaker with exposure to light; and (3) they become stronger when the cortex is restained. These signals could be derived from dye-stained neurones and glia, and although it would be interesting to know the relative contributions of these two populations, this information is not critical as both neurones and glia exhibit preferences for orientation and ocular dominance²⁹.

A second issue concerns the observation that cortex stained with NK2367 exhibits an increased absorption at 700–740 nm associated with increased activity. The direction of the change is opposite to that reported for NK2367 in studies of invertebrate neurones³⁰, heart muscle²⁰, and skeletal muscle^{31,32}. However, the voltage-dependent spectral changes of this dye are known to be species-dependent³⁰, and dye molecules bound to skeletal muscle sarcolemma and transverse-tubule membranes exhibit voltage-dependent absorption changes of opposite direction³². Finally, the prolonged staining period required to allow dye to percolate through the pia and upper layer of cortex may alter the dye-membrane interaction such that the action spectrum might invert²¹. In this regard, one reason NK2367 works better than other dyes tested lies in its ability to penetrate pia, which entails some degree of membrane permeability.

By visually inspecting cross-sections of freshly stained monkey cortex we determined that NK-2367 diffuses ~0.5 mm beneath the cortical surface. Cells lying any deeper than 0.5 mm do not stain and so presumably do not contribute to the optical signals. Although cells in the upper zone make some contribution, the amount is difficult to gauge, and vagaries of light scattering further complicate the issue. These uncertainties are not critical as long as the properties under investigation (ocular dominance and orientation selectivity) are aligned in depth and the optical results correlate with electrophysiological recordings. **Orientation selectivity and ocular dominance.** Given the newness of the technique reported here it seems inevitable that technical limitations will become more apparent with future developments. It is possible, for example, that our results are affected more than we allow by small blood vessels and by blobs (where orientation selectivity is weak). More work is clearly needed on these issues; and for the moment we can only stress the close correlation between our optical, anatomical, and electrophysiological results. With these limitations in mind, therefore, we present here the best conclusions that we can reach with regard to cortical organization.

The model of Braitenberg and Braitenberg³³ suggests that cortical regions responsive to different orientations might be organized, clockwise and anticlockwise, in a pinwheel fashion about various distributed foci. Although there are many places where this is not seen, in those where it is, the rapid changes in preferred orientation (which we call fractures) might serve merely to make adjustments in the overall map. It is difficult to see, though, how this arrangement could produce the open-looped network of fractures apparent in Figs 5b and 6b. Given the prominence of these fractures, it seems likely that they would correlate with (and possibly be derived from) some anatomical constraint. Hubel and Wiesel⁷ suggested that continuous shifts in orientation might provide an economic means of wiring up many cells with different orientation selectivity and overlapping receptive fields. Along these lines it is easy to envisage a developmental mechanism whereby neurones express some innate tendency to shift orientation preferences in their neighbours. If such a mechanism operated over ranges of 0.5–1 mm, it could lead to the condensation of stable aggregates, each with their own internally consistent map. The space constant of 0.5–1 mm could originate from local dendritic arborizations, which extend about this far.

As a slight variation of this theme, specific connections or neurones might organize the small ordered map within each module. These connections might then compete for territory and thereby produce the highly regular size that is apparent for the domains in Fig. 6b. This possibility is supported by a striking similarity between the fractures we observe and the lattice-like patterns that Rockland and Lund produced in monkey striate cortex by making large injections of horseradish peroxidase³⁴. Given the overlap between fractures and blobs in Fig. 6d, this possibility is also suggested by the finding that horseradish peroxidase injected into blobs is transported, sometimes asymmetrically, to other blobs³⁵. In this regard, it is perhaps significant that the blobs in macaque striate cortex are not usually circular and frequently extend, like fracture segments, down ocular dominance column centres.

Apart from their involvement in the anatomical and developmental constraints listed above, how might a modular design facilitate image analysis? One appealing notion is that it compartmentalizes visual space. Each module might examine a small part and reach some conclusion regarding depth and edges; the overlap between ocular dominance and orientation gradients within each module underscores this possibility. There is a problem, however, in that most modules are not large enough (by a factor of ~0.3) to accommodate all orientations. It is possible that the compartments either do not need complete sets of orientation, or, if such compartments do exist, that each may contain more than one module. On the basis of receptive field

sizes and scatter, Hubel and Wiesel⁷ estimated that a point in visual space affects the firing of cells within a 1–2-mm block of cortex. Needless to say, a block of this size could include several of the continuous domains that are cell modules.

Within a module, orientation changes continuously (to a good approximation, linearly) along a single axis. The simplest explanation for this arrangement is that it satisfies some sort of packing constraint; that is, the need to represent all orientations evenly within a given distance. Given the relatively broad orientation tuning (20–60°) for most cells, it also ensures that cells responding to a particular edge will be grouped together, which might enable some sort of local averaging to improve the reliability of detection. But why should the orientation preference remain constant along the orthogonal axis? Is there really a need for such redundancy, or is there some other response parameter (such as position, edge length or spatial frequency) that varies

along this axis? We cannot answer these questions yet, but they should influence the direction of future research and the answers should contribute significantly to our understanding of the striate cortex.

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LETTERS TO NATURE

Test of the gravitational lens hypothesis for the quasar pair 1146+111B,C

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It has recently been suggested¹ that the quasars 1146+111B,C, separated on the sky by 2.6 arc min, are actually two images of a single object produced by an extremely massive gravitational lens. The basis for this suggestion is the similarity in redshifts (within 100–200 km s⁻¹) and spectral features. Here we report differences between the two spectra in a hitherto unobserved range, suggesting that the two images are in fact distinct quasars located close together in space.

Close pairs of quasars are of interest in connection with both gravitational lensing, in which case the two images originate from the same object, and physical clustering, when the two images are due to distinct but spatially close quasars with similar redshifts. At separations of several arc seconds it is generally assumed that quasar pairs of similar redshifts and spectra are due to gravitational lenses, although even some of these could be physical pairs: the physical clustering of quasars may be similar to that of galaxies today², and separations of 5–10 arc s correspond to 30–60 kpc at redshifts of ~1–3 (for Hubble con-

stant $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and deceleration parameter $q_0 = 0$), not uncommon in clusters and groups of galaxies. Pairs at arc-min separations have generally been thought to be distinct objects, although the possibility of gravitational lensing has occasionally been raised on these distance scales as well, particularly in the context of cosmic strings^{3–6}.

Distinguishing between the two effects can be difficult for several reasons: redshifts of broad emission lines are difficult to measure accurately and can differ substantially from the true systemic redshifts⁷; spectra of different quasars tend to be broadly similar; and some spectral differences can be present even in gravitationally lensed images, because of differential extinction by intervening material or intrinsic temporal variations combined with differential time delays along the two lines of sight⁸. However, definitive tests of gravitational lensing can be made, for example, by comparing the magnitude variations of the two images, or the redshifts of narrow forbidden lines arising in the host galaxies.

The quasars 1146+111B,C, separated by 2.6 arc min, have been known for several years to have similar redshifts⁹ ($\Delta V \approx 100\text{--}200 \text{ km s}^{-1}$), and they have been included in lists of possible physical pairs². Recently, on the basis of the similarity in redshifts and spectra, it has been suggested that this pair may be produced by a gravitational lens¹. If so, it would be most unusual, as the large angular separation would imply an extremely large mass for the lensing object.

To test this interpretation, we have observed this pair in the red wavelength region, to look for similarities or differences between corresponding spectral lines in that unexplored range.

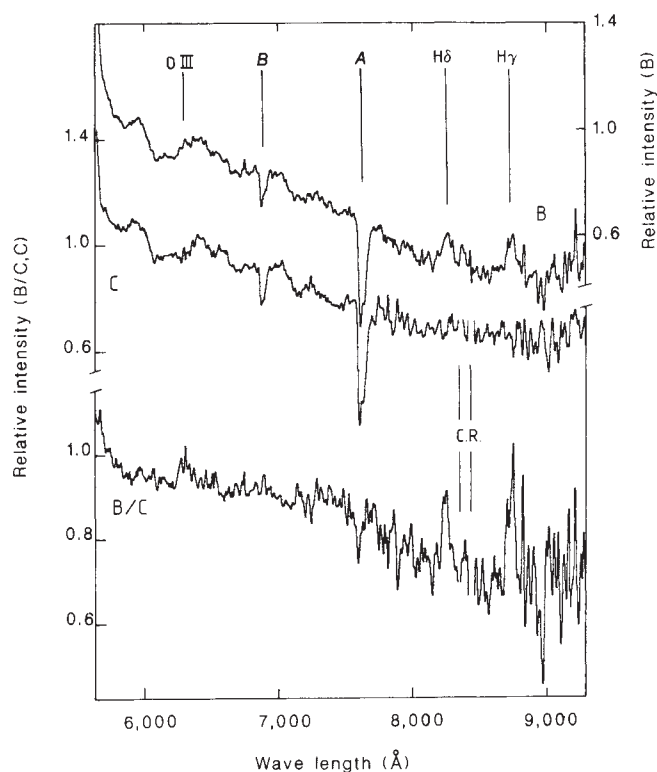


Fig. 1. Spectra of the quasars 1146+111B and C, and their ratio (B/C). The positions of the redshifted ($z=1.0115 \pm 0.0003$) emission lines of H γ , H δ and O III in 1146+111B, the atmospheric A and B bands, and two cosmic-ray events (C.R.) are indicated.

The observations were made in the period 14–16 April 1986, using the European Southern Observatory faint object spectrograph and camera (EFOSC) on the 3.6-m telescope at La Silla. A red grism providing a dispersion of 270 Å mm^{-1} was used with a slit width of 1.5 arc s, resulting in a spectral resolution of 18 Å (full width at half-maximum) throughout the wavelength range 5,600–9,300 Å. Both objects were observed simultaneously. Standard flat-fielding, sky subtraction, and wavelength and intensity calibration techniques were applied, and the resulting spectra are shown in Fig. 1.

Many features are common to both spectra, particularly the ubiquitous Fe II multiplets¹⁰. There is, however, a significant difference: the H γ 4,340 Å and H δ 4,102 Å emission lines are prominent in the spectrum of B and not seen in that of C (H γ equivalent widths of $\sim 26 \text{ Å}$ in B and $< 4 \text{ Å}$ in C). The ratio of the two spectra reveals a third possible difference, an emission feature in the spectrum of B at 6,301 Å, probably the O III, 3,133 Å Bowen fluorescence line which is sometimes seen in quasar spectra.

In view of these spectral differences, it seems unlikely that the two objects are different images of one quasar. Even allowing for possible intrinsic variations and path-length differences, it is improbable that the hydrogen lines could have varied by a factor of 6–7 relative to both the continuum and the Mg II emission line over the suggested¹ delay time of $\sim 10^3$ yr. The spectra are different, and the most straightforward interpretation is that the objects are two different quasars. The similarity in redshift is not at all surprising for a physical pair: there are enough close pairs (in redshift and projected separation) in the Véron catalogue¹¹ that the probability of at least one of them having a redshift difference $\leq 100 \text{ km s}^{-1}$ is essentially unity. There is another quasar pair, of separation 1 arc min, which was also suggested to be due to a gravitational lens¹², but closer

scrutiny revealed significant spectral differences in that case also¹³. In the absence of any compelling evidence to the contrary, therefore, we conclude that 1146+111B,C are simply two separate quasars located close to each other.

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Natural ventilation of the Paintings Room in the Altamira cave

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The Altamira cave (Santillana del Mar, Santander, Spain) is famous for the ceiling of one of its chambers, the Paintings Room, which is decorated with palaeolithic paintings. However, the massive influx of visitors resulted in deterioration of these rupestrian paintings and the cave was closed in 1977 to determine both the causes and the maximum number of visitors that could visit the cave without putting the paintings at risk^{1–3}. The natural ventilation of the Paintings Room is one of the most important factors in formulating the maximum occupational index for visitors to the cave. The emission of carbon dioxide and water vapour by visitors inside the chamber is directly proportional to the number of visitors and the time spent in the room. By ventilating the room, these components should be removed from the air within a short period of time, thus returning the chamber to the prevailing conditions before visitors were allowed in. We report here variations in the ²²²Rn concentration in the air of the Paintings Room which we use as a quantitative index of the natural ventilation existing in this chamber. We carried out parallel studies of the temperature at different points in the cave and the evolution of the carbon dioxide concentration in the air of the Paintings Room, and hence established the maximum number of people per hour that should be allowed to visit this chamber.

Radon-222 is a noble gas of the radioactive series of ²³⁸U, an element that occurs in rocks in the Earth's crust with a concentration between 2 and 5 p.p.m. (parts per 10⁶). Because of its gaseous nature and its greater concentration within the Earth, radon escapes through the interstices of the soil to the atmosphere, with an exhalation rate of $\sim 1 \text{ atom c}^{-2} \text{ s}^{-1}$. When this exhalation occurs in places with little ventilation, the radon concentration of the air may be high.

The ²²²Rn concentration of the air of the Altamira cave was measured by scintillation cells with a capacity of 500 cm³, in which a vacuum had been created down to a pressure of 50 torr. These scintillation cells were made of transparent plastic and

Table 1 Mean monthly ^{222}Rn concentration in the air of the Hall and Paintings Room; and the natural ventilation rate in the Paintings Room

Month	Location	^{222}Rn concentration (pCi/l $^{-1}$)	Ventilation rate (m 3 h $^{-1}$)
February 1983	Hall	47 $\pm$ 3	10.3 $\pm$ 1.6
	Paintings Room	76 $\pm$ 4	
March 1983	Hall	149 $\pm$ 8	9.2 $\pm$ 1.3
	Paintings Room	159 $\pm$ 8	
April 1983	Hall	162 $\pm$ 8	6.9 $\pm$ 1.0
	Paintings Room	171 $\pm$ 9	
May 1983	Hall	159 $\pm$ 9	1.0 $\pm$ 0.2
	Paintings Room	185 $\pm$ 12	
June 1983	Hall	43 $\pm$ 4	13.4 $\pm$ 2.1
	Paintings Room	67 $\pm$ 5	
July 1983	Hall	6 $\pm$ 1	20.3 $\pm$ 3
	Paintings Room	27 $\pm$ 3	
August 1983	Hall	13 $\pm$ 2	16.9 $\pm$ 2.4
	Paintings Room	37 $\pm$ 5	
September 1983	Hall	16 $\pm$ 2	17.9 $\pm$ 2.6
	Paintings Room	38 $\pm$ 5	
October 1983	Hall	37 $\pm$ 5	16.4 $\pm$ 2.3
	Paintings Room	58 $\pm$ 6	
November 1983	Hall	117 $\pm$ 7	5.1 $\pm$ 0.8
	Paintings Room	143 $\pm$ 8	
December 1983	Hall	173 $\pm$ 9	5.7 $\pm$ 0.8
	Paintings Room	180 $\pm$ 9	
January 1984	Hall	177 $\pm$ 9	9.3 $\pm$ 1.2
	Paintings Room	181 $\pm$ 9	

their internal walls were covered with a film of SZn(Ag) . The emission of α radiation by the radon ($t_{1/2} = 3.8$ days) contained in the air filling the cell leads to this radiation falling onto the film of SZn(Ag) , where it produces fluorescence. The light thus emitted can be detected using a photomultiplier tube and the pulses produced can be recorded.

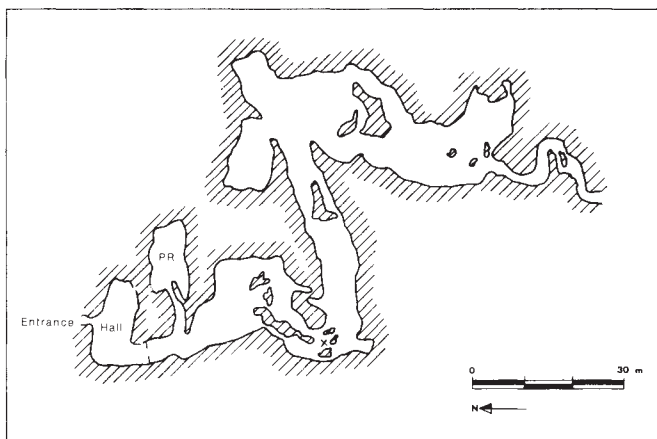
Because two of the short-lived radon daughters, ^{218}Po and ^{214}Po , are also α emitters, their contribution to the total counting supplied by an air sample increases with time until a radioactive equilibrium is reached between the radon and the two daughters. This equilibrium is reached 3 h after the sample is taken only if, initially, radon gas was present in the air sample. Therefore, to calculate the radon concentration, we carried out a 10-min counting 3 h after the collection of the air sample assuming that there was a radioactive equilibrium between the radon and its daughters. The number of counts obtained from the scintillation cell when empty was subtracted from each measurement resulting from fluorescence of the SZn(Ag) .

The ^{222}Rn concentration was measured in two chambers within the cave: the Hall chamber, located at the entrance to the cave; and the Paintings Room (see Fig. 1). Measurements were made three times per week over a period of 1 yr between February 1983 and January 1984. From these results, we calculated the monthly average radon concentrations (Table 1).

To calculate the natural ventilation in the Paintings Room from the radon concentrations, we used the simplified model proposed by Wilkening⁴, which is based on the fact that the temporal variation in the radon concentration (C) in the air of this chamber can be expressed as the sum of the production caused by radon exhalation from the rock surfaces, the radioactive decay and the radon losses resulting from the natural ventilation:

$$\frac{dC}{dt} = E \frac{S}{V} - \lambda C - \frac{Q}{V}(C - C_h) \quad (1)$$

where E is the radon exhalation rate, S and V the surface and volume of the Paintings Room, respectively, Q the ventilation rate in this chamber, λ the radon decay constant and C_h the radon concentration in the Hall chamber.

**Fig. 1** Map of Altamira cave. PR, Paintings Room.

In stationary conditions, and assuming that $Q \approx 0$ when the radon concentration reaches its maximum value $C_{\max}$ (in our case 196 pCi l $^{-1}$), then

$$E \frac{S}{V} = \lambda C_{\max} \quad (2)$$

We can calculate the ventilation rate as

$$Q = \lambda V \frac{(C_{\max} - C)}{(C - C_h)} \quad (3)$$

To apply this model to the experimental values obtained in the Altamira cave, we calculated the natural ventilation in the Paintings Room. Table 1 shows that values obtained for the ventilation rate are low throughout the whole year. Taking into account the volume of the Paintings Room, $\sim 330 \text{ m}^3$, it would take ~ 16 h to totally renew the air in the chamber by natural ventilation in July, the period of maximum ventilation rate, and ~ 330 h in May, the period of minimum rate.

At the same time as measuring the radon concentration, we studied two variables that may be related to the ventilation rate: air-temperature differences between the Paintings Room and the Hall chamber and the carbon dioxide concentration in the air of the Paintings Room.

The Altamira cave has a single entrance. In such a static cave, ventilation currents are principally caused by convective air movements that basically depend on the temperature gradients between the different points into the cave. In the Altamira cave, the air temperature differences between the Paintings Room and the other chambers, with the exception of the Hall chamber, are always very small⁵. Temperature differences between the air in the Paintings Room and that in the Hall chamber are appreciably caused by the influence of the outside temperature on the latter so convective interchanges can be expected.

Figure 2 shows the monthly mean values obtained for the ventilation rate in the Paintings Room and the temperature differences between the air of the Paintings Room and the air of the Hall chamber throughout the measurement period. The correspondence can be observed between the annual variations in both ventilation in the Paintings Room is basically determined by convective processes between these two areas.

The carbon dioxide concentration in the air of the Paintings Room has its origin from the gas dissolved in the infiltration waters that flows into this enclosure. In the same way as the radon gas, the carbon dioxide concentration in the Paintings Room is eliminated by natural ventilation as the air interchanges with the outside atmosphere. The experimental measurement of the carbon dioxide concentration was carried out using an infrared analyser connected to a continuous trace graph plotter.

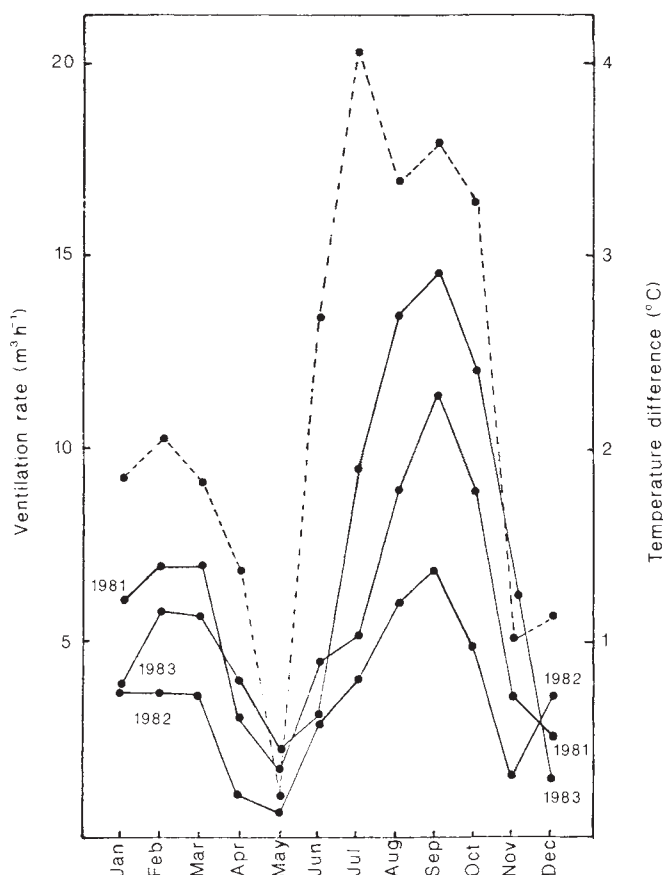


Fig. 2 Variations in the ventilation rate (dashed line) and the temperature differences (solid lines) between the Paintings Room and the Hall chamber.

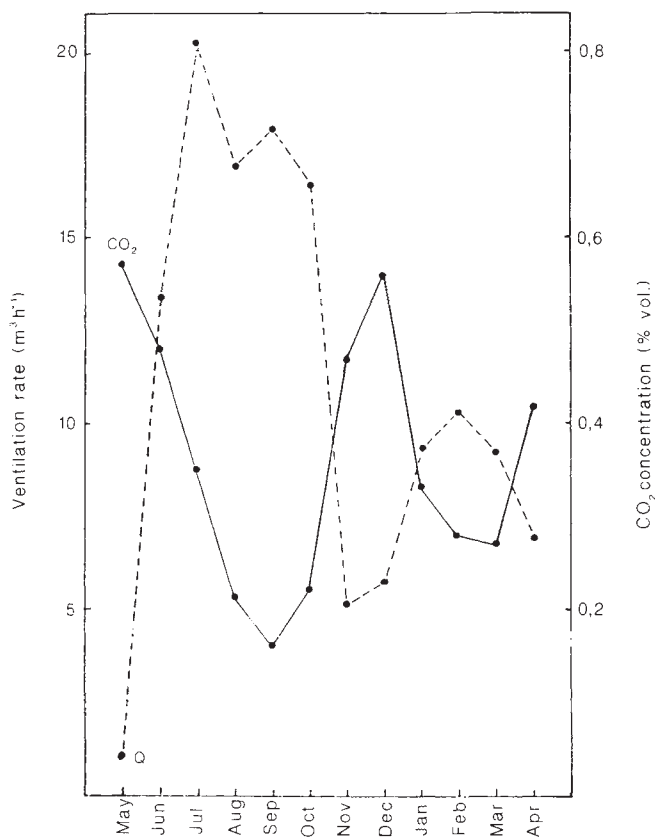


Fig. 3 Variations in the ventilation rate (dashed line) and carbon dioxide concentration (solid line) in the Paintings Room.

Figure 3 shows the ventilation rate calculated previously together with the monthly mean carbon dioxide concentrations measured in the Paintings Room. The inverse relationship between both variables consistent both with the variations in the temperature differences between the Hall chamber and the Paintings Room and with the fact that natural ventilation is the major factor affecting changes in the carbon dioxide concentration.

Taking into account that a person exhales an average carbon dioxide volume of $\sim 171 \text{ h}^{-1}$ (ref. 6) our results show that the maximum number of people that could visit the Paintings Room for a period of one hour each day in the summer months without raising the carbon dioxide concentration higher than that seen in May are 43, 74 and 80 visitors in July, August and September, respectively.

Our results indicate a weak natural ventilation in the Paintings Room throughout the whole year. The ventilation rate shows minimum values in May and November and maximum values in the summer months. These results agree with the estimations obtained from the temperature differences between the air of the Paintings Room and that of the Hall chamber and of the carbon dioxide concentration in the air of the Paintings Room. The minimum radon concentration and maximum ventilation rate, found in July, match the maximum absolute value of the temperature difference between the Hall chamber and the Paintings Room and the minimum carbon dioxide content in the air of the latter, found in the same month. In the same way, the maximum radon concentration and minimum ventilation rate recorded in May match the minimum absolute value of the temperature difference between the Hall and the Paintings Room and the maximum carbon dioxide concentration measured in this month.

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Global distribution of peroxyacetyl nitrate

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Nitrogen oxides (NO_x) have a central role in the chemistry of the atmosphere, especially in key processes relating to ozone, hydroxyl-radical (OH) and acid formation¹⁻⁷. High reactivity of NO_x (lifetime of 0.5-2 days) precludes hemispheric-scale transport and it has been proposed that non-methane hydrocarbons present in the troposphere can transform NO_x into its organic forms principally as peroxyacetyl nitrate (PAN)^{8,9}. PAN is highly stable in the colder regions of the middle and upper troposphere and can provide a mechanism for NO_x storage and transport. Once transported, PAN and its homologues can easily release free NO_x in warmer atmospheric conditions. PAN is probably ubiquitous and its concentrations could exceed those of NO_x in clean tropospheric conditions¹⁰⁻¹². Here we present the first view of the global distribution of PAN based on extensive shipboard and aircraft measurements. PAN is more abundant in the Northern than in the Southern

Hemisphere and in the continental than in the marine troposphere. In contrast to its behaviour in polluted atmospheres, PAN mixing ratios in winter greatly exceed those in summer. These measurements provide a basis for assessing the significance of PAN as a reservoir of NO_x and for extending and validating reactive nitrogen chemistry theory in the troposphere.

Details of the measurement techniques for PAN and light hydrocarbons (LHC) used in the present observational program have been described elsewhere^{13,14}. An overall accuracy of $\pm 20\%$ is estimated for the PAN measurements. We determined the hemispheric PAN distribution in surface air aboard the US Coast Guard vessel *Polar Star* while travelling from Seattle, Washington (48°N), southward to Puerta Arenas, Chile (48°S), between 11 November and 28 December 1984. All PAN and LHC measurements were made over the ocean 50 miles or more away from the coast on a daily hourly schedule from 08:00 to 22:00 local time. Figure 1a shows the inter-hemispheric PAN profile. PAN levels in the Northern Hemisphere are highly variable, ranging from <10 p.p.t.v. (parts per 10^{12} by volume) to some values >70 p.p.t.v. A sharp decrease towards the Equator is evident. Mean daily surface PAN levels in the Southern Hemisphere are only ~ 5 p.p.t.v., compared with the average Northern Hemisphere level of 38 p.p.t.v. The relatively high concentrations encountered at latitudes 28°N and 35°S are attributable to air masses of continental origin. Figure 1b shows the hemispheric profile of selected light hydrocarbons measured earlier on a similar North-South Pacific track¹⁴. Figure 1 shows that the PAN latitudinal distribution is similar to that of the alkanes, but is unlike the more uniformly distributed propene. The theoretical assertion that light alkanes (and NO_x) are probably the most important precursors of PAN, and therefore their hemispheric gradient is translatable into a PAN latitudinal gradient, is at least partially supported^{8,9}. Although reliable NO_x data are not available, it is possible to conjecture that in the lower marine troposphere, NO_x levels are extremely low (10–30 p.p.t.v.), and probably of comparable magnitude in the two hemispheres^{15,16} (also B. Ridley, unpublished data). Although PAN can hydrolyse relatively rapidly in sea water (half-life ≈ 15 min at $\text{pH} = 8.3$)¹⁷, the extremely low partition coefficient suggests that oceans are, at best, a minor sink.

Aircraft measurements to an altitude of 10 km above sea level (~ 250 mbar) were made aboard the instrumented NCAR/NSF Beechcraft King Air research aircraft. Flights were conducted in the summer of 1984 over the Colorado-Wyoming area (19–28 July; 40°N) and over the eastern Pacific (12–22 August; 40°N); and in the winter of 1985 over the eastern Pacific (12 February to 4 March; 40°N). A total of 130 flight hours were logged. Each flight lasted 5–6 h to allow for reaching ceiling altitude and for collecting a maximum number of data points during ascent and descent. Figure 2 gives examples of typical vertical PAN profiles measured during day ($\sim 11:00$ – $17:00$) and night ($\sim 22:00$ – $04:00$) in fair weather conditions. The differences between the profiles measured during ascent (solid line) and descent (dashed line) are indicative of the variability associated with region and time of day. Figure 2 shows that PAN is preserved at night with concentrations that can be comparable to the daytime values. An important feature of these data is the indication that PAN concentrations in stable weather conditions tend to increase with height, which supports the proposed theory for *in situ* PAN synthesis⁸. PAN concentrations along the summer profile in Colorado (Fig. 2a) vary between 100 and 200 p.p.t.v., whereas those along the summer profile off the California coast (Fig. 2b) are much lower, varying between 20 and 50 p.p.t.v. Observations showed that during unsettled weather (for example, when a cold front passed through the region on 27 July), PAN values over Colorado temporarily approached those measured over the Pacific. There is a great deal of structure and variability in PAN concentration in the atmosphere, especially during winter (Fig. 2c). Aircraft observations of temperature and wind obtained during the February 1985 flights

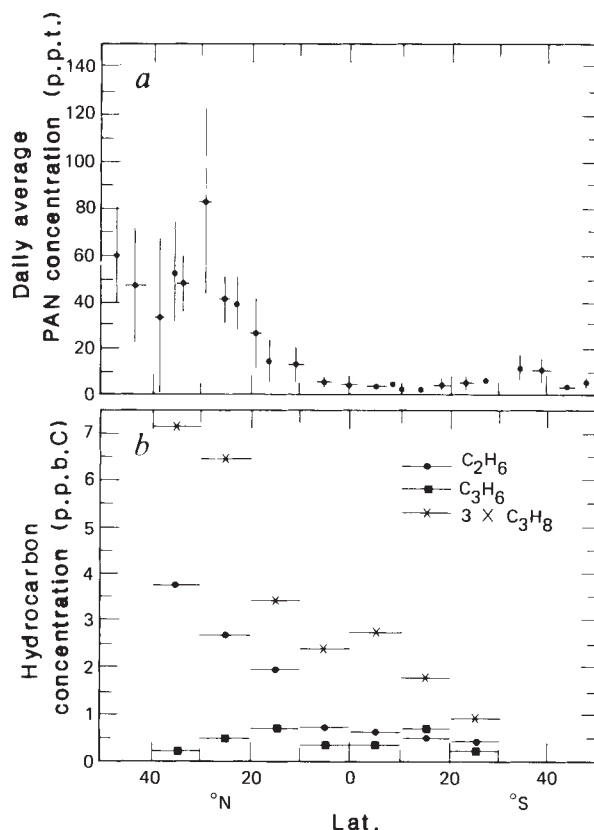


Fig. 1 Inter-hemispheric profile of surface-air concentrations for PAN and selected non-methane hydrocarbons. a, PAN data obtained from measurements aboard the US Coast Guard vessel *Polar Star*. The horizontal line segments represent the daily averaged PAN concentration in p.p.t.v. over the latitude (time) interval during which measurements were made. The standard deviation of the daily measurements ($\pm 1\sigma$) is indicated. b, Hydrocarbon data from ref. 14 obtained over the eastern Pacific Ocean during the period 30 November–21 December 1981 (p.p.b. C = 10^{-9} v/v carbon). ●, Ethane; ■, propene; ×, 3 × propane.

showed no significant variations in the vertical temperature profiles but did indicate a different wind direction and speed in the lower and middle troposphere for each of the five research flights. For the two profiles of Fig. 2c, the winds changed from north-north-west at 35–40 knots (21–22 February) to easterly at 25–30 knots (27 February). More research is needed to explain whether this is attributable to regional anthropogenic effects, to chemistry, meteorology or to all of the above.

Figure 3a,b shows composite vertical profiles of PAN concentration for the various time periods and geographical locations of the aircraft flights. Composite profiles are obtained by averaging the day and night measurements collected during stable, uniform air mass conditions. In Fig. 3a, three salient features of PAN abundance are evident: (1) in the Pacific during summer (August) and winter (February), PAN concentrations in the upper troposphere are higher than in the lower troposphere—this feature may be evident also in the summer (July) profile for Colorado except that the lower troposphere shows relatively large concentrations, possible because of continental or regional anthropogenic influences; (2) during summer, PAN concentrations throughout the continental troposphere are significantly larger than throughout the marine troposphere; and (3) PAN concentrations over the Pacific in winter are considerably higher than in summer.

The relatively high upper tropospheric PAN concentrations are consistent with PAN's extreme stability in this region, due

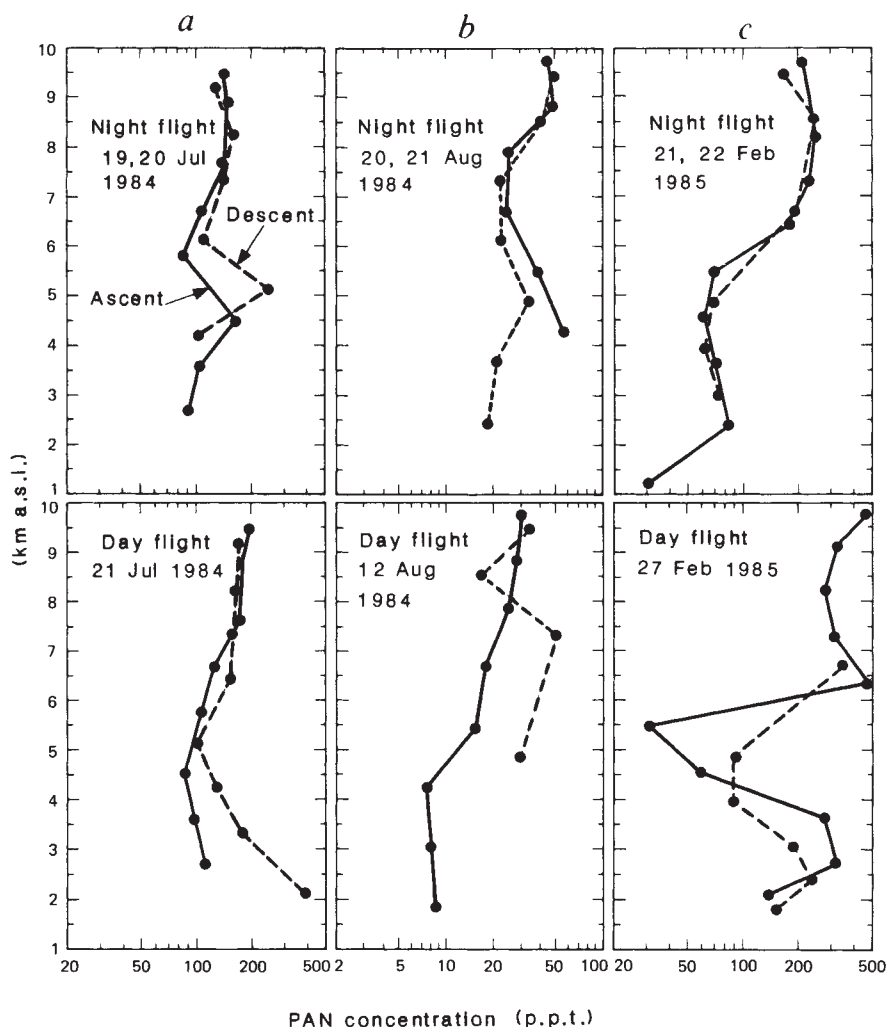


Fig. 2 Ascent (solid) and descent (dashed) vertical profiles of PAN to 10 km above sea level. Night and day profiles are shown for: *a*, the Colorado July 1984 flights; *b*, Pacific August 1984 flights; *c*, Pacific February 1985 flights.

principally to colder temperatures^{8,9}. Also, there seems to be a greater free NO_x availability in the upper troposphere^{16,18} and this would facilitate PAN formation. The continental PAN abundance of 100–200 p.p.t.v. is almost five times higher than its marine abundance during the same season (summer). This difference is even more significant when one considers that the continental troposphere was observed to be 2–4 K warmer than the marine troposphere. A 4 K increase in temperature can reduce PAN half-life by a factor of two because of the strong temperature dependence of the PAN dissociation rate [$K = 1.95 \times 10^{16} \exp(-13,543/T) \text{ s}^{-1}$]. A rational explanation must depend on the availability of non-methane hydrocarbons (NMHC) and NO_x precursors. The second feature of interest in Fig. 3a is the large increase in PAN concentration off the California coast between August 1984 and February 1985. If these profiles are representative of summer and winter, respectively, PAN levels in winter over the eastern Pacific Ocean could be nearly five times higher than in summer. This wintertime enhancement of PAN abundance is completely out of phase with what is normally encountered in polluted atmospheres, where highest values occur in summer¹⁹. The temperatures throughout the troposphere during the February 1985 aircraft flights were 8–10 K lower than those in August 1985. This temperature reduction alone could increase PAN stability by a factor of 5–6 and is likely to be an important part of this seasonal behaviour. Figure 3b shows measurements for the February 1985 California flights averaged for locations both over the ocean and ~50 km inland from the coastline. In the lower troposphere, the PAN concentrations over land are consistently higher than over the ocean. In the upper troposphere, the differences are nominal.

In addition to PAN, measurements of PPN (peroxypropionyl nitrate) were also made. PPN was frequently below our detection limit because of its very low abundance. However, during the Colorado flights it was possible to measure PPN along with PAN. Figure 3c shows composite profiles for PAN and PPN based on the July 1984 Colorado flights. The shape of the vertical PPN profile is similar to that of PAN, but its abundance is only ~5% of PAN.

Figure 4 shows the composite profile of two surrogate hydrocarbons, propane and ethane, which were measured as part of a C₂–C₅ hydrocarbon analysis conducted during the same flights. Figure 4a,b shows that although the hydrocarbon abundance over the continental troposphere is greater than that over the marine troposphere, it is so by only a factor of two or less. No reliable NO_x data from these two regions have been published. A comparison of recent NO and NO₂ data from the Pacific troposphere (B. Ridley, unpublished data) and the earlier less reliable NO_x data from this continental tropospheric region¹⁸ suggest that the latter NO_x levels are significantly higher. The concurrent higher abundance of both NO_x and NMHC precursors over the continental troposphere may be responsible for this large PAN gradient. Measurements of PAN along with NO_x and peroxyacetyl radical precursors (such as acetaldehyde and acetone) are required to reconcile adequately the observed PAN distributions. The winter NMHC abundance is also higher (Fig. 4a, c), probably because of reduced wintertime removal rates (lower OH). Global models which currently do not include NMHC–PAN chemistry point to the possibility of higher winter NO_x for the same reason⁵. However, a great deal of this extra winter NO_x seems to be contained in the form of PAN and is unavailable for photochemical participation²⁰. Figure 4d shows

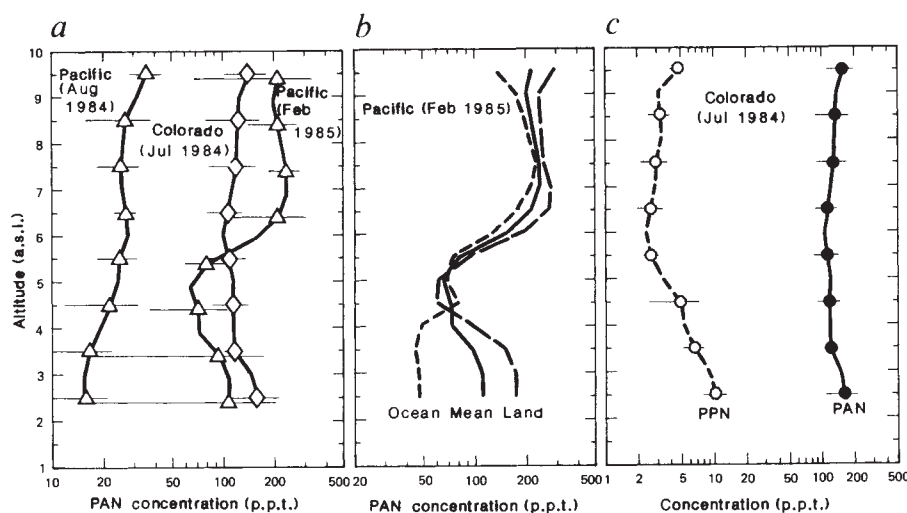


Fig. 3 Composite vertical profiles of PAN and PPN. *a*, Comparison between composite profiles ($\pm 1\sigma$) from Colorado for July 1984, and from the Pacific Ocean off the northern California coast for August 1984 and February 1985. The February profile is shifted slightly in the vertical for clarity. *b*, Mean PAN profiles observed over the ocean and 50 km inland during February 1985. *c*, PPN and PAN profile measured over Colorado.

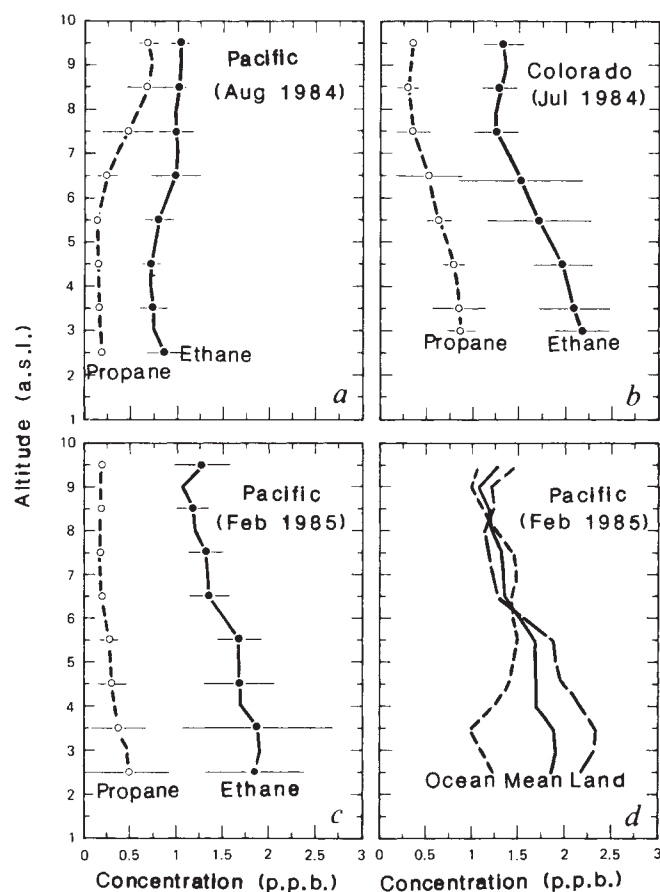


Fig. 4 *a*–*c*, Composite vertical profiles of ethane and propane. *d*, Ethane concentrations observed over the Pacific Ocean and 50 km inland during the February 1985 flights.

ethane data for the February 1985 California flights averaged for locations over the ocean and over land. These profiles can be compared with those for PAN in Fig. 3*b*. In the lower troposphere, both the ethane and PAN concentrations over land are consistently higher than over the ocean. In the upper troposphere, land/ocean differences are nominal.

Our free tropospheric PAN measurements point to the following important features: (1) PAN concentrations in the Northern Hemisphere are significantly greater and more variable than in

the Southern Hemisphere; (2) PAN concentrations show evidence of increase with height; (3) PAN concentrations in winter are higher than in summer; (4) PAN is more abundant in the continental troposphere than in the marine troposphere, at least in the summer; (5) there is a reasonable association between PAN levels and its NMHC precursors; and (6) PAN concentrations in the free troposphere are large and may exceed those of free NO_x . All of these observations are in qualitative agreement with the theory that background NMHC/ NO_x precursors can tie up significant quantities of NO_x as PAN, especially in the colder regions of the upper troposphere^{8,20}. Preliminary modelling results suggest that photochemical models which do not include NMHC–PAN chemistry may significantly overpredict the availability of free NO_x and HNO_3 in the troposphere, with consequent important implications for tropospheric ozone production²⁰. Simultaneous NO_x and carbonyl precursor data are needed to explain the PAN behaviour. Future increases in atmospheric NMHC and NO_x abundance, either due to increased emissions or falling hydroxyl radical concentrations²¹, may enhance the role of PAN in the troposphere. Indeed, this may be one of nature's ways of regulating the photochemistry of the troposphere.

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Geometry of accretion and oceanic thrusting of the Spong tang Ophiolite, Ladakh-Himalaya

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Ductile mylonitic shear zones, which in ophiolitic peridotites overprint high-temperature ductile flow, have been commonly associated with intra-oceanic thrusting, if situated at the base of the peridotites^{1,2}. In fewer cases, such shear zones have been related to the vicinity of transform faults cutting the spreading ridge³⁻⁹. In the Spong tang nappe, one of the few well preserved Himalayan ophiolite slabs, obducted southward from the Indus-Tsangpo suture zone, two successive mylonitic ductile shear events in the peridotites have been observed. The first can be related to a transform fault, the second to intra-oceanic thrusting, resulting in the superposition of two peridotite sheets^{10,11}. Poor development of the crustal sequence also indicates the presence of a transform fault, and/or to a very slowly spreading ridge. Here a three-dimensional geometry of the accretional and successive oceanic environment in the context of the eastern Neo-Tethys is proposed.

The peridotites in the high Zaskar mountains¹²⁻¹⁴ have been mapped and studied with increasing accuracy in the past decade^{10,11,15-20}. The true ophiolitic units overlie tectonically a very heterogeneous series of volcanosedimentary mélange²¹. The complete assemblage has been thrust over sedimentary series of the Zaskar unit (Fig. 1a)¹⁵⁻¹⁹.

The peridotites are harzburgitic tectonites affected everywhere by high-temperature ductile flow (S1). Two tectonic units are superposed by a mylonitic shear zone. The upper unit consists of lherzolitic harzburgite, whereas the lower one represents more depleted facies expected originally at a higher level of the sub-oceanic upper mantle.

Few cumulates are present at the east end of the lower unit (Fig. 1b, d). Ultramafics (wherlites with pyroxenitic layers) predominate over gabbros. Two measurements of the magmatic layering show it to be rather flat (Fig. 1c).

Pegmatitic gabbro and pyroxenite dykes of decimetric to metric width in the harzburgites just west of the cumulates represent magma from the cumulate magmatic chamber injected into host rock that is still hot.

Dyke swarms with a NW-SE mean orientation for each of the four observed swarms (Fig. 1b, c) intrude the cumulates. They are the only sheeted dykes observed in the Spong tang nappe.

Isolated diabase dykes a few metres wide with chilled margins, indicating their intrusion into cold host rock (~400 °C, ref. 22) are common throughout the harzburgites of the lower unit (Fig. 1c).

The high-temperature plastic flow which is linked to the ascent of the upper mantle and consequent partial fusion below a spreading centre²¹, is ubiquitous in the peridotites. The kinked olivine grains indicate activation of the (0kl) (100) 'pencil glide' of olivine at 1,000-1,200 °C and relatively low deviatoric stress^{23,24}. The orientation of measured S1 foliation planes shows a random distribution.

The dunitic banding, gabbroic schlieren as well as all ultrabasic dykelets are affected by this deformation, whereas the diabases, and the pegmatitic gabbro and pyroxenite dykes are not, showing their intrusion in the upper mantle later than the plastic flow.

The dispersion of the plastic flow structures may be explained by: (1) originally random orientation, indicating poorly-developed, slow or irregular mantle flow and the absence of a

well defined spreading axis, at least locally; (2) frequent reorientation of the S1 structures by the following deformations, as observed at a small scale (see refs 10, 11 for details).

A second ductile deformation affected the harzburgitic tectonites in distinct shear zones. Mylonitization starts from the grain boundaries of the kinked olivine and lamellar orthopyroxene (Fig. 2a), and finally affects the whole rock, leaving only small relict islands of the high-temperature plastic flow (Fig. 2b). This mylonite forms under high deviatoric stress and at 700-1,000 °C, together with the amphibolite facies metamorphism frequently observed in the accompanying metamorphic sole¹. It is also in accordance with the proposed oceanic environment²⁵.

In the Spong tang peridotites, the chronology of the two successive episodes of mylonitic shearing can be established according to the dykes they affect: the first (S2) deforms the pegmatitic gabbro dykes, coeval with the final crystallization of the cumulates, but not the isolated diabase dykes. In some cases, shear zones develop more intensely in and around these dykes, rather than in the adjacent harzburgite. This has been explained by shearing immediately following the injection of these dykes^{4,5,10,11}.

The second shear event also affects isolated diabase dykes within the shear zone, and transforms them to foliated green amphibolite, indicating a temperature of ~500 °C. It follows their intrusion into cold host rock, and thus postdates the first mylonitic shear event S2. Deformation textures in the two fine-grained olivine mylonites are very similar, but some serpentine is involved in the S2' shear zones (Fig. 2c), indicating

Fig. 1 (Opposite and page 594) a, Location of the Spong tang Klippe in western Zaskar. b, Structural map of the Spong tang ophiolite, showing the four major vertical shear zones S2 (1-4) in the west and upper unit with the observed dominating shear sense, some of the minor shear zones in the lower unit (5-7), frequently associated with gabbro dykes, and the intra-peridotitic thrust. For each shear zone the total of the observed lamellar pyroxenes in the dominant direction and in the opposite one is indicated, following the method of Reuber *et al.*²⁸. Z, Zaskar unit; VS, volcano-sedimentary sole; hbs, serpentinized harzburgite in the mélange; hb1, harzburgites of the lower unit; dun, dunitic; cum, cumulates; gab, gabbro dykes; dyk, diabase dyke swarms; hb2, harzburgites of the upper unit; S2', intra-peridotitic thrust; Φ 1, thrust of peridotites over mélange series, Φ 2, thrust of the whole Klippe over Zaskar units. c, Stereogram of the poles of diabase dykes; big dots, dyke swarms (four measurements); small dots, isolated dykes (23 measurements); squares, deformed isolated diabase dykes (two measurements) within the intra-peridotitic thrust, stars = cumulate layering (two measurements). d, Simplified cross-section along a-b, symbols are as in a. e, Stereograms of the poles of the mylonitic foliations and lineations associated showing the transform shear S2 in the upper unit (measurements of 60 foliations and 24 lineations), the intra-peridotitic thrust S2' (measurements of 26 foliations and six lineations), and the transform shear in the lower unit (measurements of 47 foliations and 13 lineations). In the upper unit, the squares represent centrimetric zones of especially intense shear, in the lower unit they represent S2 shear associated to the gabbro dykes. f, Stereograms of the poles of the pegmatitic gabbro and pyroxenite dykes (gab, 46 measurements: A, six pegmatitic pyroxenites; B, eight pegmatitic pyroxenites with a gabbroic centre; C, 16 leuco-gabbro dykes; A to C from the section on the west bank of Photang; D, leuco-gabbro dykes from other places between Photang and Central Spong); of the foliations S1 (measurements of 50 foliation planes in the upper, 55 in the lower unit, 22 lineations in the upper, 26 in the lower unit); compare with the early ultrabasic dykes (dun, dunitic banding, nine measurements in the upper, 16 in the lower unit, opx, orthopyroxene dykelets, eight measurements in the upper, 12 in the lower unit; px, websterite dykelets, two measurements in each unit; gb, gabbroic schlieren, one measurement in the upper, four in the lower unit; large symbols, upper; small symbols, lower unit). Stereograms c, e, f are equal angle projections of the lower hemisphere. Localities may be differentiated as follows: 1, west of Spong; 2, central and upper Spong; 3, Spong/Photang ridge; 4, south-east, Photang.

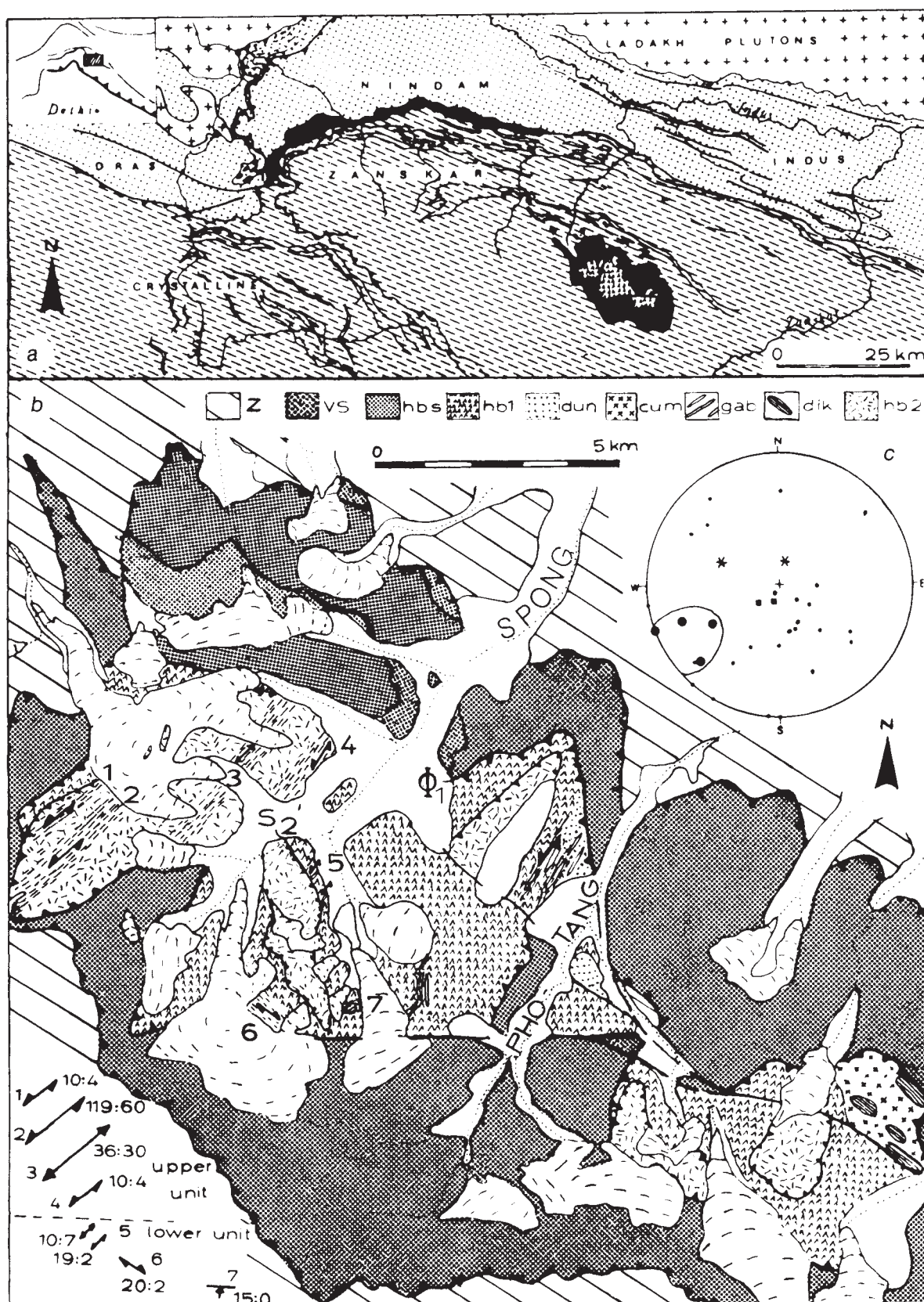


Fig. 1a/b/c

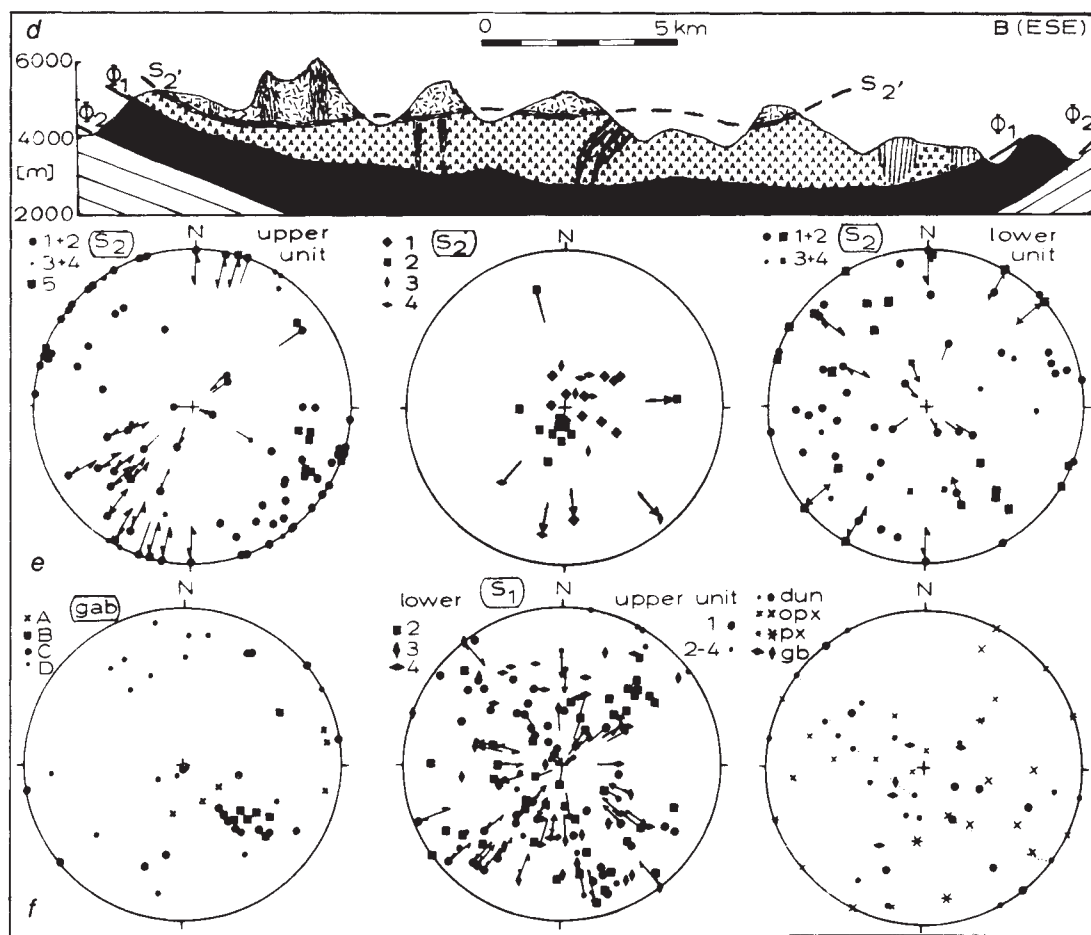


Fig. 1d/e

deformation at conditions proximate to the olivine-serpentine transformation, that is again at a temperature around 500 °C. The small temperature increase between dyke injection and shearing, can be produced by shear heating. However, this temperature is at least 200 °C too low for plastic creep in olivine²⁵, and the deformation involves olivine re-crystallization in a serpentine matrix, rather than olivine creep, a similar result to that obtained by E. Rutter (ref. 26 and personal communication).

The orientation of these shears is different. The first event, constitutes four main vertical shear zones between 50 and 600 m wide, totalling 1.6-km of sheared harzburgite within a 4-km wide zone west of the upper unit. Originally, this zone may have continued farther west beyond the actual outcrop. These main shear zones strike around N 40° E (Fig. 1b, d), and the associated ductile shear is predominantly sinistral, as shown by lamellar orthopyroxenes, interpreted directly in the field^{27,28}. From west to east the sinistral shear is progressively accompanied by pure flattening, as the relative number of orthopyroxenes indicating dextral shear increases (Fig. 1b).

In the lower unit, several minor shear zones add up to an additional ~500 m width of sheared harzburgite dispersed over 5 km. Their orientation as well as the observed shear sense become progressively dispersed and less important towards the east (Fig. 1e), as expected at the edge of a mega-shear zone.

The second mylonitic shear S2' is concentrated in one main shear zone of sub-horizontal orientation (Fig. 1e), and a width of 20–70 m. This zone, marking the limit between the upper and the lower peridotite units, can be followed throughout the Spong tang Klippe (Fig. 1e, d). It is most visible in the mountain north of Spong glacier, where a sheared diabase dyke is coated

with white magnesite (Fig. 3). The few lineations and the little information available on the shear sense indicate, however, a predominance of south- to southeastward thrusting of the upper unit over the lower one (S2: 11 lamellar orthopyroxenes, Fig. 1e).

The present data constrain the accretionary geometry of the Spong tang ophiolite. In the absence of palaeomagnetic data, rotation with respect to a vertical axis cannot be traced, and the data are treated as if no such rotation occurred during or after obduction. A small rotation with respect to an horizontal axis will improve the internal kinematic coherence of the model proposed as follows.

The dykes of the dyke complex are, in general, considered to be parallel to the ridge orientation²². In the absence of a true dyke complex, the mean orientation of the dyke swarms indicates a NW-SE orientation for a poorly developed ridge (N 140° E).

The S2 vertical shear zones may be linked to a transform fault zone continuing farther west beyond the actual outcrop. The transform fault should be parallel with the major shear zones, thus trending NE-SW (N 40° E).

The flat S2' shear can be related to intra-oceanic thrusting in a NNW-SSE direction. Thus, for considerations of accretionary geometry, the upper unit can be restored towards the NNW of the lower one.

The proposed ridge and transform orientations are nearly perpendicular, and the predominantly sinistral shear of the transform zone results in a dextral offset of the ridge segments.

This idea is consistent with the poorly-developed cumulates in the vicinity of the transform fault, which limits the cumulate magmatic chamber in the west. This observation does not exclude a normal crustal section farther east beyond the Spong tang Klippe. Predominantly ultrabasic cumulates at the edge of

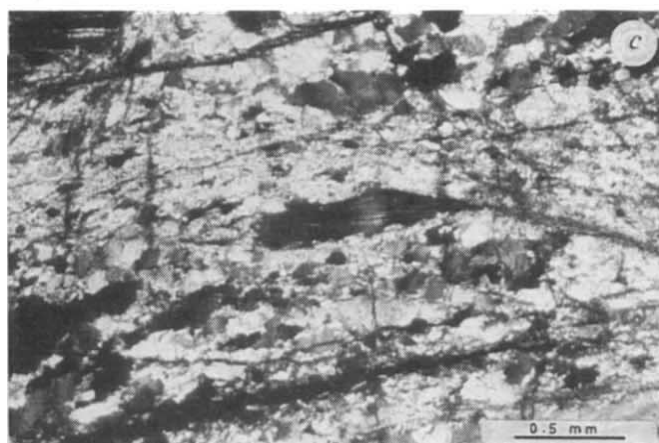
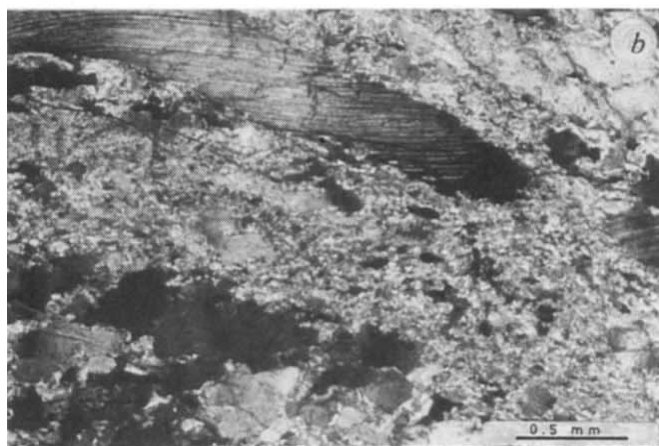
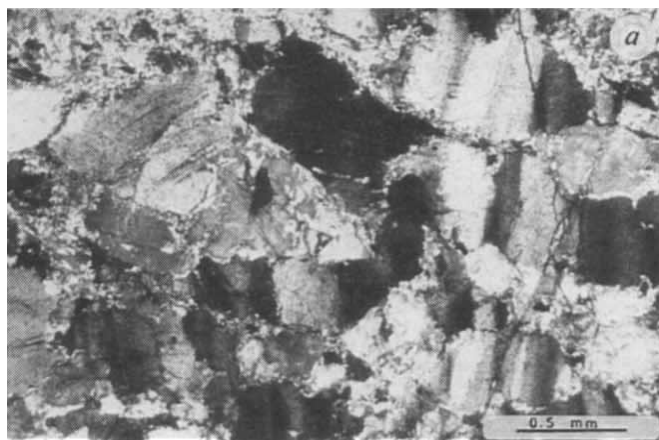


Fig. 2 *a*, Start of mylonitization at the grain boundaries of kinked olivine and lamellar pyroxene porphyroclasts. *b*, Complete mylonitization with few relic porphyroclasts, S₂ shear. *c*, Intense mylonitization including some serpentine, of the S₂' shear. All are photomicrographs, cross-polarized light.

a transform fault have previously been described in a very similar set-up for the Antalya ophiolite (Turkey^{4,5}). The high content of primary clinopyroxene in the harzburgites of the western part and the upper unit indicates again little partial fusion as would be expected in a compressive transform zone²⁹.

A rotation of 20°, less than the dispersion of the measurements for S₂' foliation planes, with the azimuth of the mean S₂' plane as the axis, is proposed to restore a thrust movement on the S₂' foliation planes (Fig. 4c). At the same time, the normal component related to the transform shear S₂ becomes inverse (Figs



Fig. 3 At the Spong glacier the intra-peridotitic thrust is underlined by the white magnesite coating of a deformed diabase dyke.

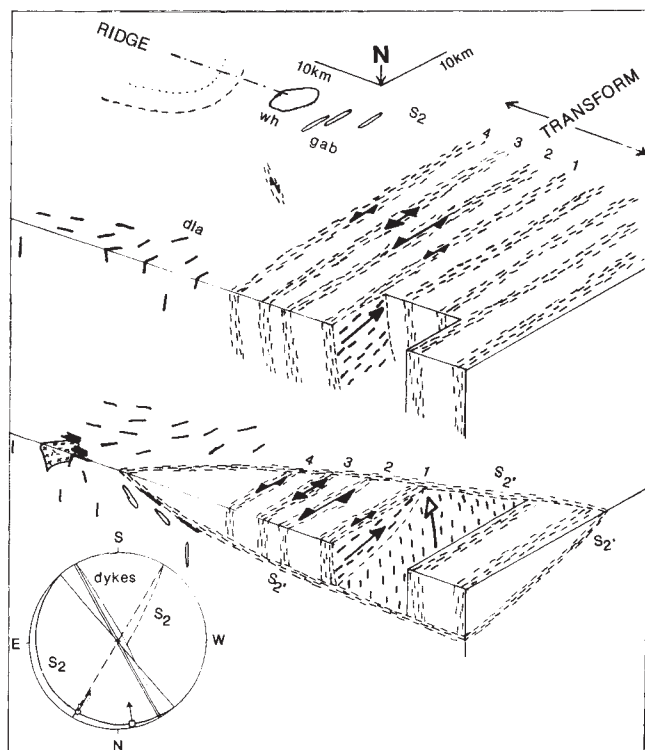


Fig. 4 *a*, Sketch of the oceanic accretion geometry before intra-oceanic thrusting, looking southward. Disposition of the ductile shear zones (1–4) at an approximately median level of the cumulate magmatic chamber (in white) possibly existing farther east of the transform fault. The observed cumulates rather originate in a small magmatic chamber (wh), nearer to the transform zone, where the pegmatitic dykes (gab) are also found, the intrusion of the isolated diabase dykes (dia) is drawn off-axis (not to scale). The true position of L₂ is shown in the inset. *b*, Intra-oceanic southward thrusting of the transform zone over the block east of the transform, the inset shows position of L₂ and L₂' lineations. *c*, Stereogram showing the circular trace of the mean position of the main planar structures and associated lineations after rotation to their proposed original position as explained in the text.

1e, 4c), which is in better accordance with the compressive aspect of this transform zone.

This is the first ophiolitic example of intra-oceanic thrusting resulting in the superposition of two peridotite units. The absence of amphibolites of volcanosedimentary origin underly-

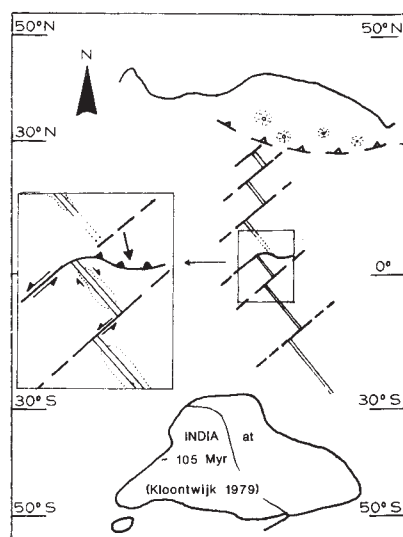


Fig. 5 Sketch of the possible geometric relations between the accretionary ridge, the transform fault and the intra-oceanic thrusting in the context of the beginning northward move of the Indian plate. Its northern boundary is stable at 30° south from the Permian to Middle Cretaceous³³, a time span which should include the phenomena described in the text.

ing the thrust peridotites^{10,11,20,21}, can be explained by: the absence of volcanism in the transform zone; the scarcity of volcanosedimentary cover due to thrusting shortly after accretion; Spongtag representing a deep-seated part of the section.

This model, which is concerned with details of the accretionary geometry, does not decide the global geotectonic environment. However, there is a strong preference for the creation of Spongtag ophiolite in the open ocean, because: transform faults and intra-oceanic thrusts are not known in back arc basins; preliminary geochemical results show mid-ocean ridge basalt characteristics for more than half of the studied samples of volcanics of the sole of Spongtag as well as from the Dras area^{30,31}.

This does not exclude the possibility that such oceanic crust travelled into the arc environment through the combined effects of spreading and subduction, and eventually became overprinted by arc intrusives, volcanics, and associated sediments³².

Applying this model to the eastern Neo-Tethys, shows us the possibility of maintaining extension along NW-SE oriented ridge segments, associated with minor dextral shear, even under the beginning of NNW-SSE compression. The latter is concordant with sinistral shear on the NE-SW oriented transform faults, possibly even beyond their true transform segment, and may initiate the first intra-oceanic thrusts (Fig. 5).

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A magnetotelluric sounding across Vancouver Island detects the subducting Juan de Fuca plate

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Magnetotelluric¹ (MT) and geomagnetic² depth-sounding measurements have been made at 18 sites (7 with remote reference³) across Vancouver Island (Fig. 1), beneath which the Juan de Fuca plate is underthrusting^{4,5}. Vancouver Island is part of the old accretionary Wrangellia terrane⁶ and is located in the central portion of a 350-km forearc region that extends from the sediment-filled trench⁷ to the Garibaldi volcanic belt⁸. Gravity⁹ and seismic refraction studies¹⁰ suggest that the descending plate has a shallow northeasterly dip of 8°-16° with perhaps higher dips beneath northeastern Vancouver Island. A high-resolution seismic reflection survey¹¹ along lines 1 and 3 (Fig. 1) located a zone of strong acoustic reflections (the E-horizon¹¹) near the top of the subducting Juan de Fuca plate at depths of 23-34 km. Our model of the broad-band MT data defines a sloping, highly conducting zone also near the top of the subducting plate at the same depths as the seismic E-horizon. The high electrical conductivity in this zone (the E-conductor) is the result of saline fluid within the pore spaces of sedimentary and mafic materials of the upper oceanic crust that have been subducted to those depths beneath Vancouver Island. These are the first data which clearly define a dipping conductive layer associated with the boundary region between converging plates; they have significant implications for thrust earthquakes and metamorphic reactions that occur in subduction zones.

The MT data were processed at each field site in real time, to evaluate data quality and, where possible, to maintain standard deviations of <5% for apparent resistivity and <±1° for impedance phase. The upper panels of Fig. 2 show the suite of apparent resistivity curves spanning six decades from 384 Hz to 1,820 s determined in the major and minor axes of anisotropy and grouped for the electric field measured approximately perpendicular (B-polarization mode) or parallel (E-polarization mode) to the strike of the continental margin. The curves in each group are similar in shape and display a decrease in amplitude at periods longer than 1 s, an indication of increasing conductivity at depth. The apparent resistivities for these two modes diverge at the longer periods (>30 s), which is charac-

teristic of data from stations on the resistive side (the crust beneath Vancouver Island) of a boundary adjacent to a more conductive structure (the seawater and oceanic crust). The vertical displacements of up to two decades along the ordinate are caused by near-surface resistive¹² or conductive^{12,13} anomalies. The average phase curves, however, have small standard deviations at periods >10 s, indicating a generally laterally uniform resistivity distribution in lower crust beneath southwestern Vancouver Island.

At site 12, the two orthogonal resistivity curves (highlighted in Fig. 2) and phases (not shown) are nearly isotropic for periods <10 s while at sites 10 and 14 the isotropy is moderate for periods <1 s. We have used an inversion procedure^{14,15} on the data for these three sites to obtain one-dimensional layered models (Fig. 3). The sedimentary materials covering the valley beneath site 14 are more conductive than the resistive bed rock with little overburden beneath sites 10 and 12. Below depths of several kilometres the crust is moderately resistive, in the range 5,000–15,000 Ωm . All models are characterized by a low-resistivity layer (the E-conductor) at depths of 23–28 km which agree ($<10\%$) with those to the seismic E-horizon (bold bars in Fig. 3). The long period divergence of the data between the two modes precludes a precise determination of the thickness and resistivity of the E-conductor with one-dimensional models. A two-dimensional interpretation is required to explain both polarization modes with the same model.

The two-dimensional model (Fig. 4) was constructed using the known conductivity and thickness of the sea water in the Pacific Ocean and in the Strait of Georgia. The parameters of the accretionary wedge beneath the continental margin west of Vancouver Island are consistent with the two-dimensional geomagnetic depth-sounding model of DeLaurier *et al.*¹⁶. Their model, as well as the seismic interpretation of Spence *et al.*¹⁰, suggest that the oceanic lithosphere/asthenosphere boundary is near 25 km depth and dips to 55 km beneath the coast of Vancouver Island. The depth to the E-conductor is determined by the one-dimensional inversion models (Fig. 3) and by the seismic E-reflector¹¹, except for that beneath the very northeastern part of the island where there is an apparent absence of deep seismic reflections. The E-conductor increases in dip from about 10° beneath the three MT sites to about 35° beneath the Strait of Georgia, consistent with gravity models⁹ and the down-dip of the tension axis from earthquake focal mechanisms¹⁷. A conductive region^{18,19} is required in the lower crust beneath mainland British Columbia to model the long-period geomagnetic transfer functions and the MT data, although its depth and resistivity are not well determined by a survey limited to Vancouver Island. The thickness and resistivity of the E-conductor were varied until good agreement ($<5\%$) was attained between the model response²⁰ and measured data at site 12. Since no attempt was made to include the near-surface structures apparent in the models of Fig. 3, a good fit to only the long period (>10 s) phases at sites 10 and 14 was obtained. At all sites, however, the geomagnetic transfer functions were predicted for periods >0.1 s.

The E-conductor near the top of the descending Juan de Fuca plate is 6 km thick with a 30- Ωm resistivity, and extends up-dip to merge with oceanic layers 1, 2 and 3. We have not investigated the response of a very thin conductor, say 1 km, since this necessitates a fine mesh in the model, resulting in unreasonably large matrices to invert. However, even a thin E-conductor must have a conductance (thickness/resistivity = 200 S) close to that in the model. Although our data do not resolve detail in the oceanic crust west of the coast, the conductance of the E-conductor is compatible with those obtained in oceanic layers 1 and 2 from seafloor controlled-source electrical sounding experiments^{21,22} and deep-sea drilling resistivity logs²³. The seismic E-reflection zone is 3–5 km thick, and Green *et al.*¹¹ suggest several possible sources for this, including layered sediments, intercalated volcanics and sediments, layered igneous basement rocks or tectonic structures induced by underthrusting.

The two-dimensional model (Fig. 4) simulates the subduction of the Juan de Fuca plate beneath the forearc region. Low resistivities in the lower crust or upper mantle have frequently been reported and are attributed to fluids filling the pore spaces²⁴ or to partial melting²⁵. The heat flow pattern is typical of forearc regions²⁶; heat flow decays from moderate values on the west coast of Vancouver Island to a minimum east of the Strait of Georgia, increasing to high values over the Garibaldi volcanic belt (T. Lewis, personal communication). Based on heat flow measurements, the temperature beneath Vancouver Island increases to $400 \pm 100^\circ\text{C}$ at the top of the E-conductor; at greater depths, within the young subducting plate, the downward displacement of isotherms suggests that this estimate is a maximum. A temperature of 500°C is more than a factor of 2 less than is required to give a resistivity of 30 Ωm for dry granites or gabbros²⁴. Furthermore, it is not sufficient to induce partial melting in wet basalts or granites²⁷. Consequently, the materials within the E-conductor beneath our sites must be porous and saturated with saline fluid.

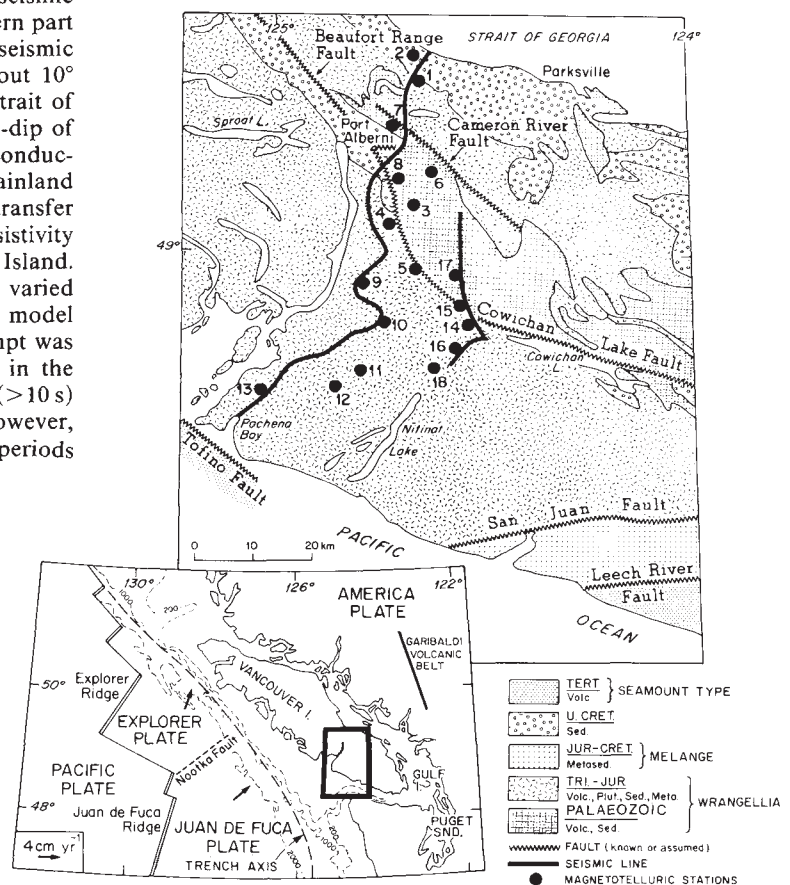


Fig. 1 Simplified geology (adapted from ref. 38) of southwestern Vancouver Island and the locations of the magnetotelluric (MT) sites near seismic reflection lines 1 (west) and 3 (east). The inset shows the location of our survey area with respect to the four plates and the pertinent convergence arrows. Bathymetry is in metres.

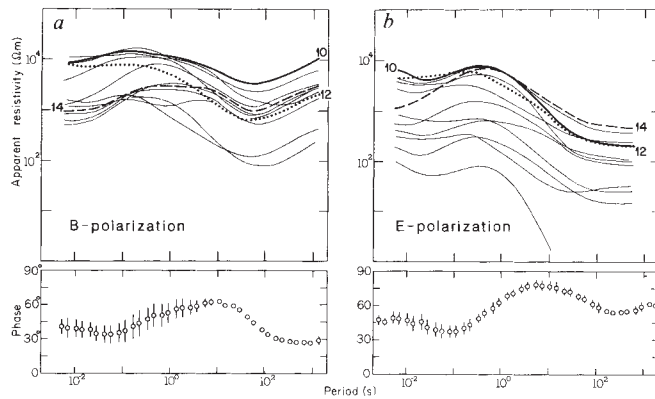


Fig. 2 The apparent resistivity curves for the southern MT sites (including remote reference sites if available). A discussion of the more complex data from the remaining sites will be presented elsewhere. The highlighted curves are for stations 10, 12 and 14 discussed in the text. Lower panels show the averaged phases associated with all the apparent resistivity curves in the corresponding upper panel. Error bars are two standard deviations in length. *a*, B-polarization mode for the electrical field perpendicular to the coast. *b*, E-polarization mode for the electric field parallel to the coast.

A simple form of Archie's law expresses the ratio of the fluid resistivity to the bulk resistivity as equal to the square of the porosity^{23,28}. If the fluid filling the pore space within the E-conductor has the salinity of sea water (3 wt% NaCl), and its resistivity at 375 °C is 0.04 Ωm (ref. 29), then the bulk resistivity of 30 Ωm leads to a porosity of 3.6%. Salinities higher than that of sea water could be expected since the solubilities of mineral species increase with temperature³⁰. Therefore, the porosity can be lower; for example, a 20-wt% NaCl solution at 375 °C has a resistivity of 0.008 Ωm and, with the E-conductor resistivity, the porosity is 1.6%. Pressure effects are negligible, as the load pressure of ~850 MPa (assuming a crust density⁹ of 2,920 kg m⁻³) is more than sufficient to maintain the liquid phase³¹ at the expected temperature at the top of the E-conductor. These porosities, an order of magnitude larger than those measured³² in the laboratory for mafic crystalline rocks, could be supported with fluid pressures equal to lithostatic. This requires impermeable boundaries above or within the E-conductor. If the fluid pressures are hydrostatic, then the rock

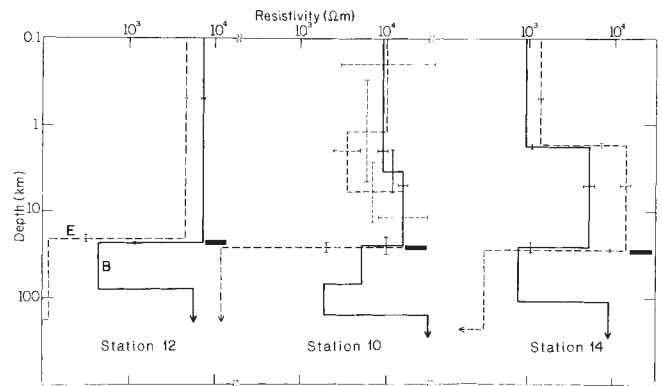


Fig. 3 One-dimensional models obtained by inversion of the apparent resistivity and phase for the E- and B-polarization data from stations 10, 12 and 14. The bold horizontal bars indicate the depths to the top of the seismic reflection zone that originates from the boundary region between the descending Juan de Fuca plate and overriding North American plate. The standard errors are determined from an eigensolution analysis³⁹.

matrix is supporting the differential pressure. As the pressure and temperature at the top of the E-conductor suggest that metamorphic reactions within the blueschist to greenschist fields³³ are occurring, dehydration²⁶ may enhance porosity³⁰ and provide a source for the fluid. The trapping of fluid within the sedimentary and mafic materials of the descending plate is another complementary source.

Our MT depth-sounding data see, beneath Vancouver Island, a highly conducting zone near the top of the subducting Juan de Fuca plate which, at the expected temperatures, suggests that a conductive fluid fills the pore space of the materials within the E-conductor. This result has important geophysical implications for active or exhumed subduction complexes. First, the low level of historic seismic activity compared with the tectonic convergence rate of the Juan de Fuca and American plates³⁴ suggests aseismic creep on a zone of lubrication, perhaps the result of high pore pressure, although it is possible that strain accumulation occurred over the period of the seismicity data³⁵. Second, the variation of the acoustic impedance across fluid-

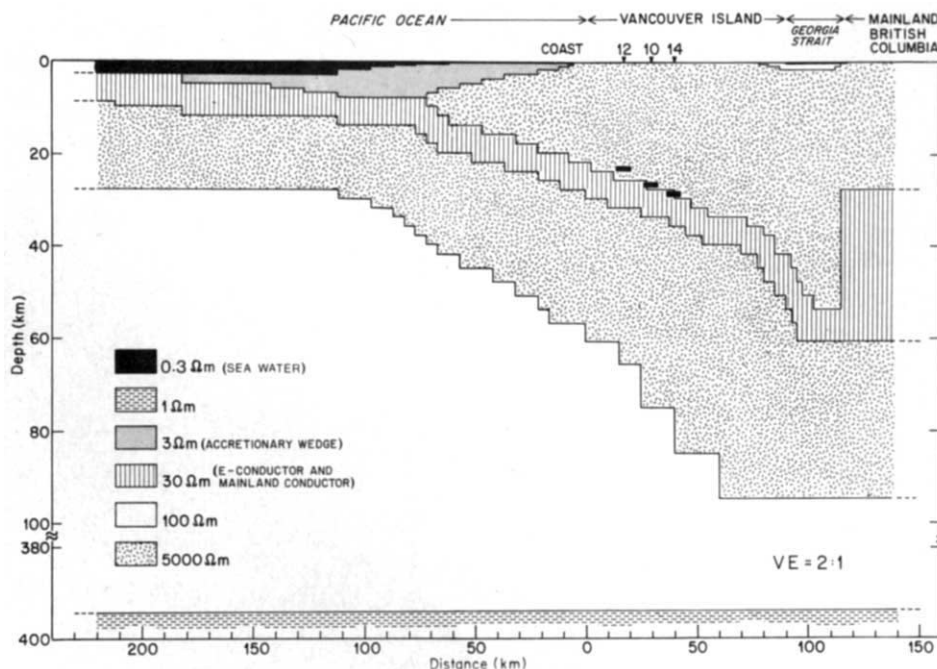


Fig. 4 The two-dimensional electrical resistivity model representing the subduction of the Juan de Fuca plate. The E-conductor (30 Ωm) is near the top of the plate. The bold horizontal bars beneath sites 10, 12 and 14 on Vancouver Island indicate the depths to the seismic E-horizon. The global decrease in resistivity⁴⁰ is represented by a 1-Ωm half-space below 390 km.

saturated porous materials³⁶ could explain the band of multiple, enhanced seismic reflections observed beneath the E-horizon¹¹. Third, the conducting zone beneath the western portion of the forearc region suggests a depth of origin for the widespread occurrences of high-pressure/low-temperature metamorphic rocks such as glaucophane schists³⁷. All these possibilities seem to necessitate the presence of fluids with pressures near or equal to lithostatic.

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Seaward extension of the East African Rift

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The continental margin of northern Mozambique is edged by a north-south trending alignment of submarine peaks forming the Davie Ridge (Fig. 1a). From earlier studies^{1–5}, the ridge has been interpreted as a remnant transform fault that controlled the late Jurassic/early Cretaceous drift of Madagascar and the opening of the Somali Basin^{6–8}. During MD40-MACAMO cruise (June 1984), 7,500 km of water gun seismic reflexion lines have been implemented throughout the region where two flat-bottomed troughs, the north-south trending Kerimbas and Lacerda deep grabens, interrupt the continental slope alongside the ridge. These grabens cut into Tertiary sediments correspond westward, to a Miocene tilting or a recent normal fault; and eastward, to an old discontinuity several times reactivated and located above an abrupt uplift of the Moho. The two grabens are separated from each other by a rigid region of thickened crust, affected by intense seismicity and supporting volcanoes. We report here that the eastern branch of the East African Rift joins the submerged grabens across the Tanzanian continental shelf (Fig. 1b).

From 9° S to 17° S, the Mozambique margin is split into three domains (Fig. 1a). (1) North of the Saint-Lazare Seamount, the continental slope is interrupted downwards by a 250-km long trough known as the Kerimbas Basin⁹. Its 20–30-km wide flat-bottom dips slightly northward (2,400–2,800 m deep). Its asymmetrical edges are cut into four segments directed either north-northwest or north-northeast. The western boundary runs alongside the foot of the uppermost slope of the margin, which is cut by gullies. To the east, the basin is bounded by the western 1,000 m and more high escarpment of the Davie Ridge, sometimes formed by steps. The top of the wide (120 km) Davie Ridge is 1,700 m below sea level and gently dips towards the Somali Basin. (2) The rugged relief is interrupted southward by two circular features: the Saint-Lazare (12° S) and Paisley (14° S)

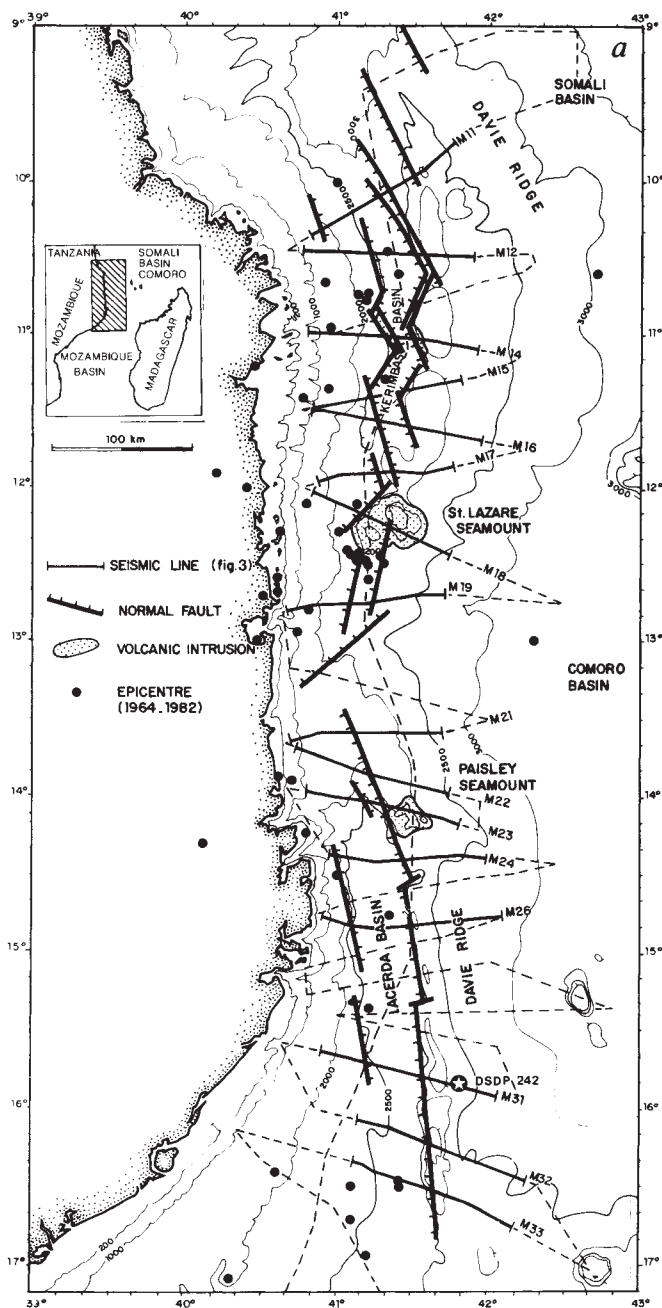
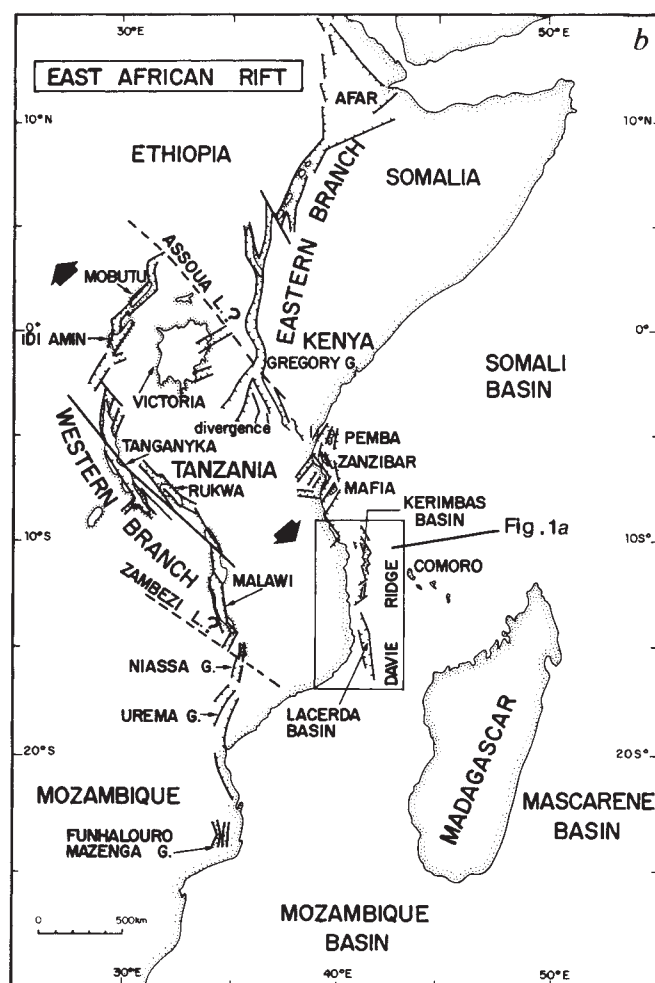
seamounts, between which the slope gently deepens towards the Comoro Basin. (3) South of the Paisley Seamount, the abrupt uppermost continental slope is cut by several canyons that terminate near the shore. The Davie Ridge is bounded by a flat-bottomed 400-km elongated depression, the 2,700-m deep Lacerda Basin. To the south both edges of the trough become more asymmetrical, as the continental slope widens and the Davie Ridge narrows and rises. Thus, the Davie Ridge culminating at 300 m deep below sea level, is rising as much as 2,300 m above the Lacerda Basin.

The relief pattern on the continental slope off the northern Mocambique is strongly related to neotectonics. Reflection seismic lines reveal that the steep elevation of the Davie Ridge with respect to both basins, appears to be a scarp produced by a normal fault. East of the Kerimbas Basin, several newly formed staggered faults (Fig. 3) cut the thick well stratified sedimentary layers (2.5 s two-way travel time). The fault scarps decrease at either end of the basin (Fig. 3a). East of the Lacerda Basin a nearly linear normal fault edges an horst consisting of probably continental basement that forms the diffracting substratum of the ridge. This horst is part of a north-south trending alignment of three parallel ridges underlain by Tertiary cover⁹. This horst has been remobilized as shown by unconformities (Fig. 3b) within the Tertiary cover east of the ridge and by upturning at the fault contact in most of Neogene layers infilling the Lacerda basin.

Within the trough, most of the latest depositions are discordant and onlap the fractured, flexured and even folded older formations (Fig. 3). These deformations are related to probable strike-slip subvertical faults. Both normal and strike-slip fractures may have originated by several breaking tectonic phases and by an oblique opening of these troughs. To the west, the basins are edged by a flexure or a normal fault, at the foot of upper slope of the margin. These troughs are thus interpreted as grabens in the central region and as half-grabens at either end.

Volcanic intrusions occur on both sides and at either end of these grabens. A dyke and a sill (Fig. 3) can be observed where the Kerimbas graben is at its narrowest. Here, an alkaline basalt bearing peridotites nodules sandwiched within middle Miocene (N14) to present (N23) haemipelagic sediments has been dredged (DR03). The size and shape of Saint-Lazare and Paisley seamounts, whose intrusive appearance on seismic lines is associated to a sharp magnetic anomaly, denote their igneous origin. Both features are interpreted as volcanoes contemporaneous with the evolution of the grabens.

Fig. 1 *a*, The northern Mozambique continental margin: the continental slope is discontinued downward by troughs (the Kerimbass and Lacerda basins), except between the Paisley and Saint-Lazare seamounts interpreted as volcanic intrusive bodies. These basins are bounded by normal faults running alongside the foot of the upper continental slope and the western scarp of the Davie Ridge. Most of the epicentres are located west of the ridge beneath grabens, south of the Saint-Lazare Seamount and near the shoreline of Mozambique. Bathymetry in metres drawn by Jean René Vanney. Dashed lines, ship tracks. *b*, The East African Rift: graben (G) and transform faults or lineaments (L) after ref. 14. The eastern branch of the East African Rift is not interrupted with the north Tanzanian divergence but extends southeastward across the Tanzanian continental shelf²⁰ and the continental slope off Mozambique. With such mapping, both branches of the rift are sigmoid shaped and of the same length. Arrows, direction of extension derived from microtectonic analysis^{14,24} and seismicity^{11,25}.



The age of Tertiary bedding within the continental margin has been inferred from correlations with DSDP 242 drilling⁵ and from core samples. The tilting running alongside the upper-slope and the Davie Ridge, the variation in thickness of sedimentary layer at either side of the ridge fault scarp, and the graben itself are likely to have originated at middle Miocene time. The undisturbed horizontal stratification within the trough, and on the other hand the undulation of seismically transparent layers of the continental rise show that the depression was already differentiated at Plio-Quaternary times. The Lacerda graben is originated from an older structure but it is also of middle Miocene age, as shown by flexures and onlaps observed under the continental rise and under the slope (Fig. 2b). But the upturning of Miocene strata by faulting denotes a later deformation occurring at Plio-Quaternary time. Faults and flexures located South of the Saint-Lazare Seamount (Fig. 2) are related

to this period of activity, however, their throw is small and have a limited influence on the morphology.

Neogene deformations of the northern Mozambique margin can be explained by the tilting of two domains located at either side of a major discontinuity below the western scarp of the Davie Ridge. Samples from a condensed and decarbonated series of late Cretaceous age have been cored on the ridge, indicating an uplift up to 1,500 m since the Maastrichtian¹⁰. On the continental shelf, emergent marginal reefs and canyon heads also indicate a period of uplift. So the deepening of troughs and the tilting of the upper slope may explain the instability of this zone where canyons and slumps occur (Fig. 3).

From International Seismological Centre data, we plotted epicentres of earthquakes related to this region and occurring between 1964 and 1982 (Fig. 1a). Most of the earthquakes are shallow and of magnitude <5. As already quoted for the East

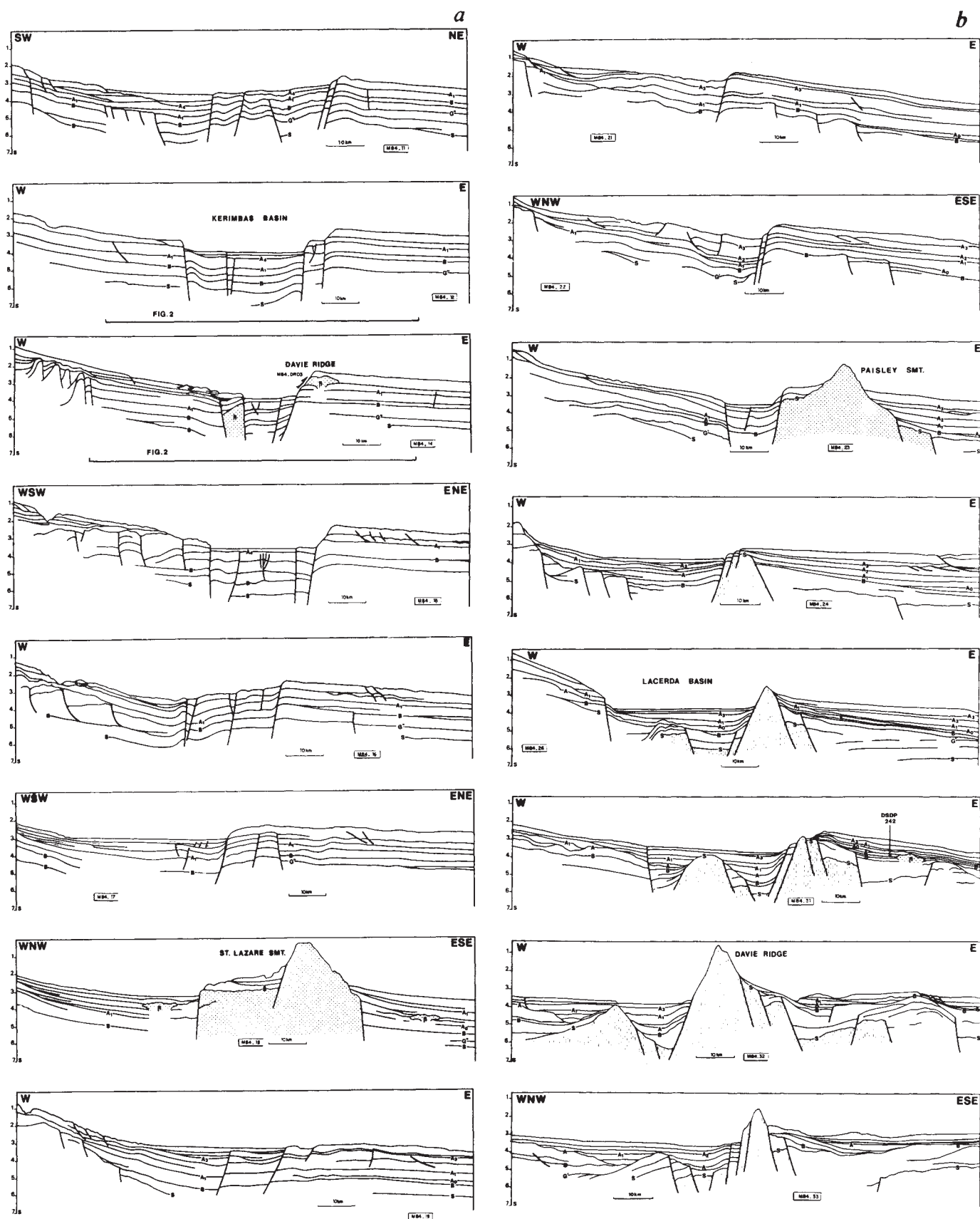


Fig. 2 Two-way travel-time seismic sections (as located on Fig. 1a) through the Kerimbas (a) and Lacerda (b) Basins. The structure formed by symmetrical faults is only shown from the median region of either basin. Only normal faults alongside the Davie Ridge extend towards the ends of the troughs. To the north, the ridge is formed by a thick well bedded sedimentary series; to the south by a horst of diffracting pre-tertiary basement later reactivated. Dotted lines, volcanic intrusive bodies; dashed lines, continental basement; S, acoustic basement; B, Cretaceous/Tertiary boundary; A, Eocene/Oligocene boundary; A₀, Oligocene/Miocene boundary; A₁₋₂, middle Miocene; A₃, late Miocene; A₄, Plio-Quaternary.

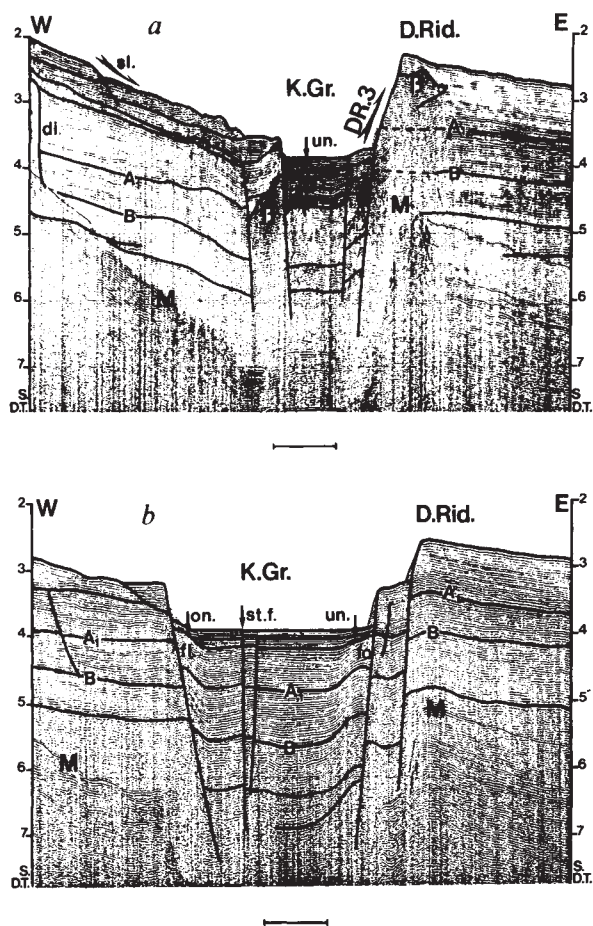


Fig. 3 Single channel watergun seismic profiles: *a*, profile 14; *b*, profile 12. Sections through the Kerimbas graben (K. Gr) and the Davie Ridge (D. Rid) exhibit the asymmetry of the troughs and associated features. β , Volcanic intrusion (dyke and sill); di., diapirism; fl., flexure; fo., fold; on., onlap; sl. slumping; st. f., strike-slip fault; un., unconformity; M, bottom multiple. Scale bar, 10 km.

African Rift¹¹⁻¹⁴, epicentres are not only located along the grabens, but also extend far away, from the grabens to the shore. Epicentres are clustered south of the Saint-Lazare Seamount where the grabens disappear. Seismicity is poorly active east of the western fault scarp of the Davie Ridge which forms a major discontinuity.

P-wave travel-time anomalies have been computed from residuals (O/C) published in ISC Bulletins (O, observed travel time; C, computed travel time from an Earth model). Having made corrections for the influence of the structure below the seismological observatories located in southern Africa and in Madagascar¹⁵, previous studies^{12,13} showed that the variation of residuals with earthquake location suggests at first glance mainly a change in the structure below the epicentre, such as a Moho-deepening of 2 km for 0.15 s residuals. Along the Davie Ridge, residuals are slightly negative (Fig. 4), except between the Saint-Lazare and Paisley seamounts, where grabens are younger or absent. We presume that residuals emphasize the presence of a thickened continental crust below this active zone that may impede the opening of the rift.

From the continent throughout the ridge, a sharp drop of residuals (-1.7 s at $41^{\circ}30'$ E) denotes initially a dramatic eastward shallowing of the Moho from 35 km below Mozambique to 12 km deep below the Comoro Basin. However, the variation of residuals with longitude does not preclude lateral inhomogeneities within the crust and the upper mantle. In the absence of deep refraction lines, some of the results quoted above are not well constrained. This discontinuity is probably

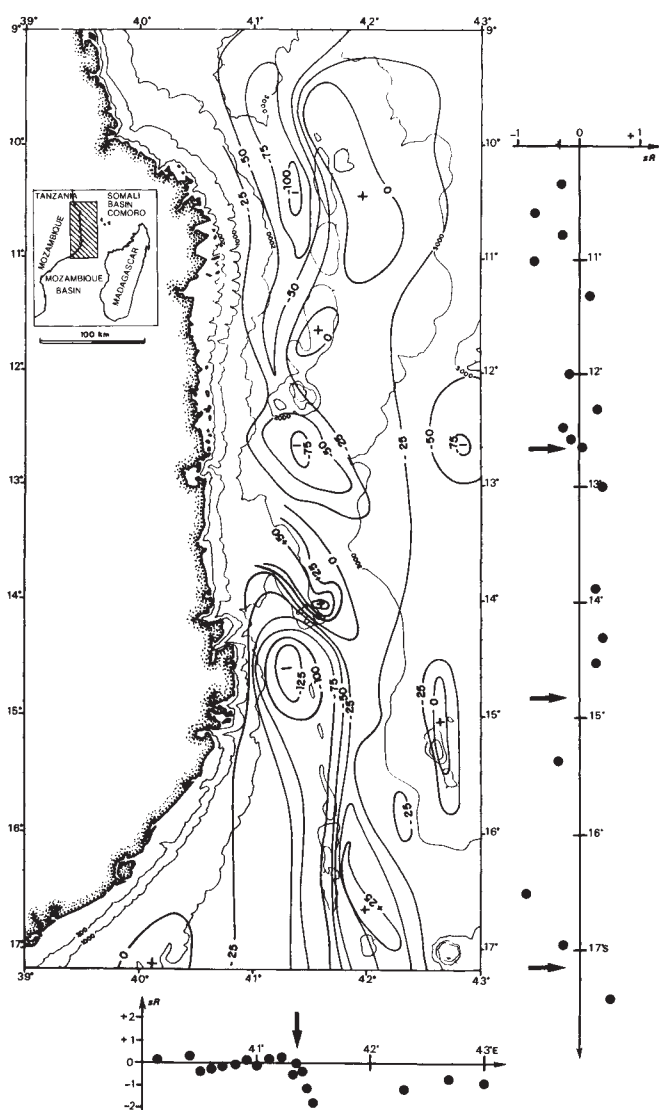


Fig. 4 Bathymetry, gravity free-air anomaly map¹⁸ and associated P-wave residual anomalies¹² alongside the continental margin off northern Mozambique. Across the ridge, the steep gradient between negative and positive gravity anomalies may be correlated with a dramatic advance of P-wave travel-times interpreted on a first step, as a sharp landward thickening of the continental crust, that is roughly superimposed over the westward fault scarp of the ridge. North-west-south-east trending discontinuities are observed between the Paisley and Saint Lazare seamounts, where both earthquake epicentres and volcanic intrusive bodies are clustered. This domain of thickened crust may be one wherein the rift is propagating. *sR* in seconds denote residuals corrected for the structure below the seismological observatories located in Africa and Madagascar.

inherited from a fossil transform fault¹⁶. Sandstone and schist samples dredged from the ridge⁴ and the lack of significant magnetic anomaly preclude an ocean/continent boundary along the western flank of the ridge and support earlier studies^{10,12,17} emphasizing that the Davie Ridge belongs to the African domain with the presence of a sharp intra-continental discontinuity governing the graben pattern and the local seismicity.

The correlation between free-air anomalies¹⁸ and bathymetry is good (Fig. 4). However, short-wavelength anomalies and high gradients may be modelled only by either upright elongated bodies culminating at shallow depth within the crust or by a dramatic change into the crustal structure. Free-air gravity values are negative over Kerimbas and Lacerda grabens, whereas they are positive over the Davie Ridge. Despite a slight eastwards offset, these gravity gradient may be associated with P-wave

travel-time anomalies and support the north-south trending of the crustal discontinuity along the grabens. Although the Kerimbass Basin is newly created, this feature and the Lacerda Basin seem to be generated from an older structure.

Between the Saint-Lazare and Paisley seamounts, north-west-south-east-trending positive and negative gravity anomalies outline transverse structures parallel to the main transform lineaments of the East African Rift¹⁴. These discontinuities not seen in the shallow fracture pattern, mark the boundaries of the thickened crust zone wherein volcanism activity and most of the earthquakes occur. This area could be a good example of a locked zone¹⁹ to propagating rifts during continental breakup.

Earlier studies¹⁴ quoted that the eastern branch of the East African Rift terminates at the North Tanzanian divergence (Fig. 1b). But seismicity¹¹ suggests the existence of a tensile zone extending the rift, on land in a north-west-south-east direction and seaward from the Tanzanian coastline (7° S) to the northern Mozambique continental slope (17° S). This active zone was not thought to be related to the continuation of troughs observed along the eastern branch of the African Rift¹⁴. Recently, Neogene basins have been revealed from seismic surveys on the continental shelf off Tanzania²⁰ and Kenya²¹ substantiating the interpretation²² that Pemba, Zanzibar and Mafia islands (Fig. 1b) are remnant horsts, reactivated in Miocene times and edged by subsident Neogene synclines. So, Tanzanian grabens would form a link between the eastern branch of the East African Rift and troughs alongside the northern Mozambique continental margin.

The western branch of the East African Rift extends also southwards as far as Cretaceous southern Mozambique grabens²³, reactivated recently and outlined by a topographic depression. So shaped, both branches of the East African Rift are sigmoidal like and of comparable extent. The distribution of the seismicity¹¹ and the rift pattern as depicted by this study,

support a deformation picture affecting the whole of eastern Africa, rather than well defined boundaries, early stage of an oceanic opening.

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Eutectoid phase transformation of olivine and spinel into perovskite and rock salt structures

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The 700-km seismic discontinuity which defines the top of the Earth's lower mantle is generally attributed to the transformation of the upper mantle (Mg, Fe)₂SiO₄ spinel into magnesiowüstite (Mg, Fe)O and (Mg, Fe)SiO₃ with perovskite structure¹. Large-volume high-pressure experiments² have provided important thermodynamical information on this transition, but its mechanisms and kinetics are still unknown. Here we report microstructural observations of the products of the transformation of (Mg, Fe)₂SiO₄ olivine and spinel and Ca₂GeO₄ olivine, effected in a laser-heated diamond anvil cell (DAC). The observations were made by transmission electron microscopy (TEM), a technique that has already proved useful in investigating the olivine-spinel transition in the DAC^{3,4}. We find that, in all cases, the transformed product has a eutectoid-like appearance (as in pearlitic carbon steels), with alternating lamellae of the rock salt and perovskite phases. The thickness and spacing of the lamellae increase with temperature until a granular structure forms. These observations suggest that the transformation starts as a eutectoid transformation, whose physical mechanisms might be as effective in the Earth's mantle as in the DAC.

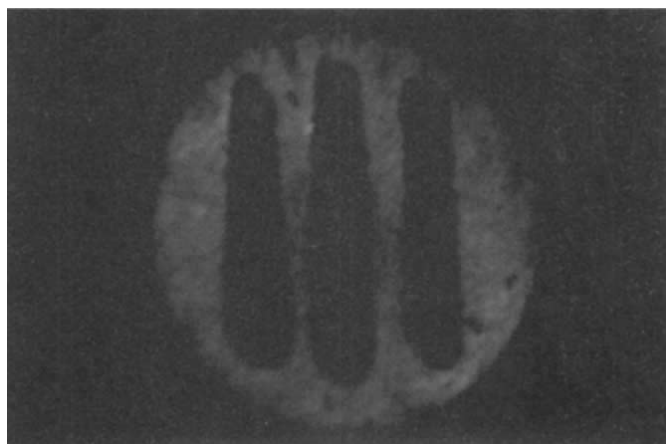


Fig. 1 Olivine in the DAC, transformed into perovskite and magnesiowüstite in the three vertical strips that have been laser-heated.

The starting material powder was placed in the 300-μm-diameter hole of a hardened nickel gasket, 200 μm thick, and pressurized in the DAC to a maximum pressure (*P*) of ~400 kbar at the centre of the cell. Pressure was measured by calibrated strain gauges on the steel members of the cell⁵, to avoid introducing ruby which could react with the material under investigation. A 60-W YAG laser beam was focused on a 30-μm-diameter spot and scanned across the sample at a speed of 100 μm s⁻¹. Three strips of transformed material, each 50 μm wide, were then produced (Fig. 1), allowing analysis of the effects of various pressure and temperature conditions. (The temperature across a strip ranged from the minimum temperature necessary to effect the transformations at the edges of the strip, up to an estimated temperature of 2,000 °C along the axis.) The two strips at the

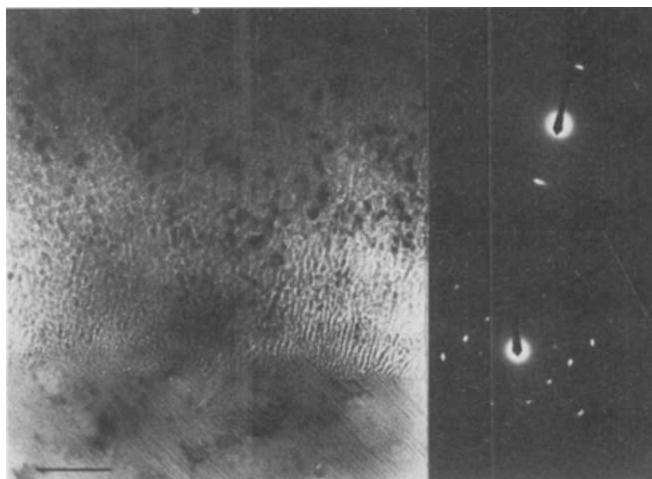


Fig. 2 Contact between sheared olivine-spinel phase (bottom) and eutectoid perovskite-rock salt structure. The dark subvertical lamellae are strongly textured magnesiowüstite (top diffraction pattern at right). Towards the centre of the strip (top) the structure becomes granular. Scale bar, 0.2 μm .

edges of the cell were at pressures too low for the perovskite-rock salt transition, but high enough for the olivine-spinel transition, whereas the centre strip was at the maximum pressure of 400 kbar in the central part of the sample. After heating, the sample was slowly decompressed, ion-beam thinned and examined by TEM at 200 keV.

In the first series of experiments, the starting material was San Carlos olivine (Fo_{88}). Examination of the edge strips confirms previous results^{3,4,6,7} on the olivine-spinel transition, showing intercalated olivine and spinel layers at the edge of the strip, giving way to polygonal grains of spinel with stacking faults at higher temperatures towards the strip axis.

At the edge of the central strip, the same sheared olivine-spinel polytype is visible (the superimposed olivine and spinel diffraction patterns show that the (100) plane of olivine and the (111) plane of spinel are parallel); towards the axis of the strip this region is abruptly replaced by another with a very regular lamellar structure (Fig. 2) which becomes coarser and eventually granular at higher temperatures towards the axis. The smallest spacing (and thickness) of the lamellae is ~ 50 – 100 Å. The dark lamellae are identified by electron diffraction as magnesiowüstite (Mg, Fe)O; the light lamellae give no diffraction pattern. By continuity, they can be shown to consist of a glass with $(\text{Mg, Fe})\text{SiO}_3$ composition: in the high-temperature region,

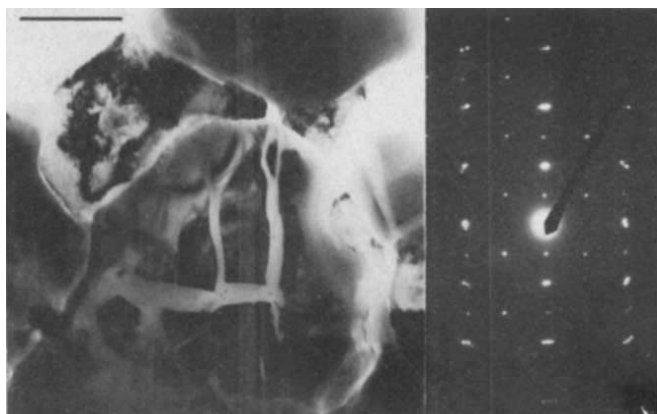


Fig. 3 Rectangular domains of $(\text{Mg, Fe})\text{SiO}_3$ perovskite before amorphization under the electron beam (diffraction pattern at right). In the top left-hand corner a grain of magnesiowüstite with a very high dislocation density is seen. Scale bar, 0.5 μm .

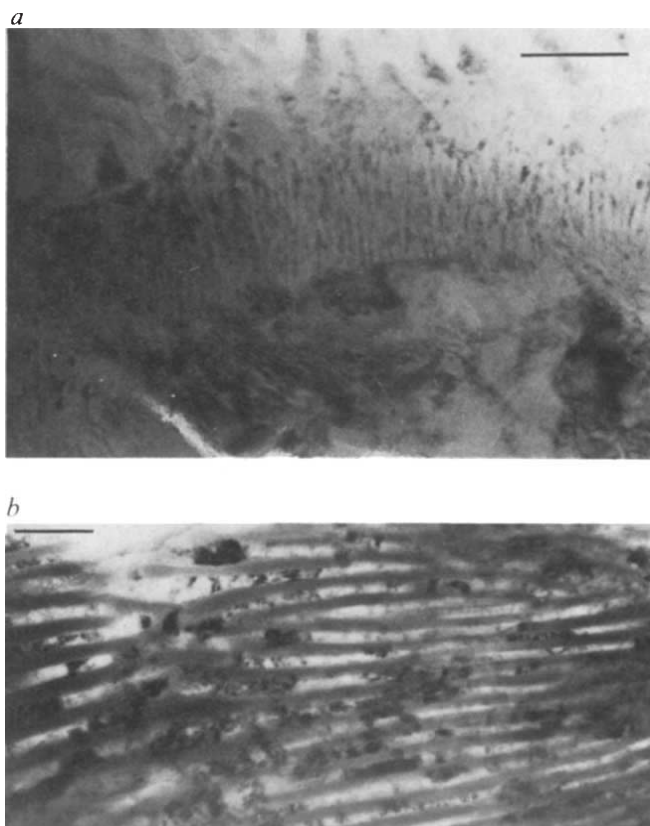


Fig. 4 *a*, Eutectoid structure of the perovskite-rock salt phases formed from *a*, spinel (bottom); *b*, Ca_2GeO_4 olivine. Towards the centre of the strip in *a* (top), the spacing of the lamellae increases. In *b*, dark bands are CaO , light bands are amorphized perovskite. Scale bars: *a*, 0.1 μm ; *b*, 0.2 μm .

similar glassy, roughly rectangular grains were produced by amorphization under the electron beam of crystalline $(\text{Mg, Fe})\text{SiO}_3$ perovskite grains (Fig. 3) whose grain boundaries are parallel to (100) planes of the ideal cubic lattice. The grains, without any dislocations, correspond to twinned domains of the GdFeO_3 -type distorted structure. Magnesiowüstite grains contain a very high density of dislocations, which accounts for the invariably dark contrast of the magnesiowüstite lamellae.

In the second series of experiments the olivine was first transformed to spinel by uniformly scanning the sample, compressed to a maximum pressure of 200 kbar, with the laser beam. The sample was then compressed to 400 kbar and the laser beam drawn across it in strips as previously. TEM examination showed that the starting material then consisted of spinel grains with the usual (110) stacking faults. The transformed material was identical to the previous one and exhibited the same lamellar microstructure (Fig. 4*a*), coarser towards the centre of the strip.

All our observations point to the transformation $(\text{Mg, Fe})_2\text{SiO}_4 \rightarrow (\text{Mg, Fe})\text{O} + (\text{Mg, Fe})\text{SiO}_3$ being of the discontinuous, eutectoid type well known in metallic alloys^{8–11}. The lamellar microstructure, typical of the transformed product, is thought to be caused by the growth of nuclei by bulk and grain-boundary diffusion in the direction of grain-boundary migration¹⁰. From the geometry of our experiments, the reaction seems to start at the centre of the strip, the grain boundaries migrating towards the edges, this direction being favoured by the internal stress arising from the decomposition⁹. The inter-lamellar spacing decreases as temperature decreases¹³. The mechanism of the formation of nuclei of $(\text{Mg, Fe})\text{O}$ or $(\text{Mg, Fe})\text{SiO}_3$ is unclear, but might involve a transient local distortion of the spinel structure into the K_2NiF_4 structure. This structure, denser than spinel, is not stable at any pressure for $(\text{Mg, Fe})\text{SiO}_4$,

but it is stable for Ca_2GeO_4 at pressures greater than 100 kbar (ref. 14); it can be described as an alternate stacking of layers with the perovskite and the rock salt structure. At ~ 200 kbar, Ca_2GeO_4 transforms into $\text{Ca}_3\text{Ge}_2\text{O}_7 + \text{CaO}$ (ref. 15). We performed with Ca_2GeO_4 the same type of experiments as with the silicate olivine, starting with Ca_2GeO_4 powder with olivine structure and compressing it to 300 kbar. TEM examination showed, for the first time, that it eventually decomposes into CaO and CaGeO_3 perovskite, also with eutectoid structure (Fig. 4b). It is therefore possible that shear and cation shuffling of the spinel structure¹⁶ might produce defects with local K_2NiF_4 structure, thus providing embryos of MgO or MgSiO_3 that can grow by diffusion until the grain boundaries start moving and creating the cellular structure.

The DAC and TEM coupled experiments on the perovskite + rock salt decomposition therefore lead to several conclusions: (1) The decomposition in the DAC exhibits all the characteristics of a eutectoid reaction driven by diffusion and grain-boundary reaction, that is, by processes that may be active in the mantle (but leading to different lamellar spacing and thickness). (2) The K_2NiF_4 structure exhibited by Ca_2GeO_4 at moderate pressure decomposes at higher pressure into rock salt and perovskite structures; a transient distortion to the K_2NiF_4 structure may have a role in the post-spinel decomposition of silicates.

(3) The high dislocation density of the magnesiowüstite may come from plastic accommodation of the 10% volume decrease on transformation; this could be the first hint that magnesiowüstite could be more deformable than perovskite in the lower mantle.

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Visualization of the dynamic instability of individual microtubules by dark-field microscopy

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It has previously been shown that two populations of microtubules coexist in a dynamically unstable manner *in vitro*: those in one population elongate while those in the other shorten and finally disappear^{1,2}. This conclusion was based on changes in the number and length distribution of microtubules after dilution of the microtubule solution. Here, we demonstrate directly that growing and shortening populations coexist in steady-state conditions, by visualization of the dynamic behaviour of individual microtubules *in vitro* by dark-field microscopy. Real-time video recording reveals that both ends of a microtubule exist in either the growing or the shortening phase and alternate quite frequently between the two phases in a stochastic manner. Moreover, growing and shortening ends can coexist on a single microtubule, one end continuing to grow simultaneously with shortening at the other end. We find no correlation in the phase conversion either among individual microtubules or between the two ends of a single microtubule. The two ends of any given microtubule have remarkably different characteristics; the active end grows faster, alternates in phase more frequently and fluctuates in length to a greater extent than the inactive end. Microtubule-associated proteins (MAPs) suppress the phase conversion and stabilize microtubules in the growing phase.

Microtubules, very dynamic structures that can rapidly assemble and disassemble^{3,4}, form a cytoskeleton that is stable in the interphase of the cell cycle and that disintegrates rapidly into tubulin subunit in the prophase of mitosis. The subunits then completely reorganize to form the mitotic spindle⁵. In the late telophase stage of mitosis the reverse process takes place, that is, the spindle microtubules disassemble and the cytoskeletal microtubules re-form. To determine the mechanism by which microtubules assemble or disassemble without changing the concentration of tubulin subunits, we observed the dynamic behaviour of individual microtubules by dark-field light microscopy. When a single microtubule is monitored, its two ends grow at different rates (Table 1) as described previously^{6,7}. However, each end independently stops growing in a stochastic manner, and then immediately begins to shorten at high speed (Fig. 2). After shortening for 1–30 s, the microtubule suddenly stops this process and starts growing again. Each end can thus exist either in the growing phase or in the shortening phase, and can alternate between the two phases in a stochastic manner.

These phase conversions at the two ends of a single microtubule occur independently, and conversions take place without any correlation among the individual microtubules on each slide in the microscope. As a result, some microtubules shorten whereas others elongate within a field of observation (Fig. 1), and no synchronized phase conversion is observed. This excludes the possibility that a cyclic environmental change in a specimen causes the switching between the two phases of microtubules. All the microtubules shown in Fig. 1 exhibit the phase changes, and >90% of the filaments in any particular observation field undergo phase conversions. The filament labelled b in Fig. 1, for example, attached to the glass surface throughout its length while filament e attached at only one point on the filament. As a result, when the direction of flow changed, filament e pivoted about its attached point whereas filament b

Table 1 Kinetic parameters of the dynamic instability

End	Growth rate ($\mu\text{m min}^{-1}$)	Shortening rate ($\mu\text{m min}^{-1}$)	Shortening length ($\mu\text{m phase}^{-1}$)	Duration time		No. of samples	
				Growing (min)	Shortening (min)	Growing phase	Shortening phase
Active	0.63 ± 0.15	7.9 ± 2.2	2.4 ± 1.9	2.9 ± 1.8	0.30 ± 0.23	54	69
Inactive	0.20 ± 0.06	15.2 ± 7.7	0.73 ± 0.35	6.0 ± 4.0	0.05 ± 0.02	32	23

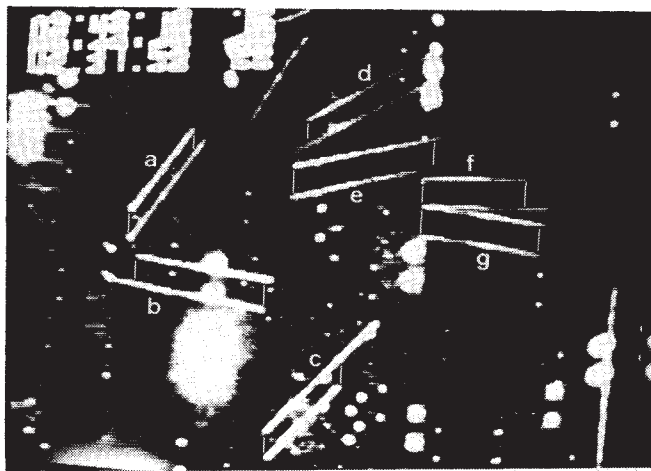


Fig. 1 Instability of microtubules visualized by dark-field microscopy. Several microtubules were attached to a glass surface so that their growing and shortening processes could be monitored continuously. Dynamic images were recorded on videotape. To demonstrate the changes in their lengths, two video images at different times (37 min 53 s and 43 min 18 s) were photographed on the same frame of a film by double exposure. The camera position was moved slightly before the second exposure so that the change in length could be seen. The two ends of a microtubule interconverted a few times between the growing and shortening phases during the time interval between the two exposures. As a result, some ends elongated while others shortened. The white lines show the shift of a corresponding point on a microtubule caused by movement of the camera position. Comparison of the images in pairs shows that the upper end of the microtubule designated as a, has shortened, the upper end of c has elongated, and so on. **Methods.** Tubulin was prepared from calf brains by three cycles of assembly-disassembly as described previously¹¹. Further purification of tubulin was performed by phosphocellulose column chromatography⁸. Assembly of a microtubule was initiated by raising the temperature to 37 from 0 °C in the tubulin solution at a concentration of 1.7 mg ml⁻¹ in buffer (90 mM PIPES, 1.8 mM EGTA, 0.9 mM MgSO₄, 2.7 mM GTP, pH 6.9). After incubation for 2 min, 4 µl of the solution was transferred onto a slide glass, mounted with a coverslip (22 × 22 mm) and observed by dark-field light microscopy. Microtubules were illuminated through an Olympus DC dark-field condenser by a Ushio 500-W high-pressure mercury arc lamp and observed with an Olympus Apo ×40 objective lens. The dynamic dark-field images were recorded on videotape using an ultrasensitive Ikegami CTC-9000 SIT video camera as described previously¹². Scale bar, 10 µm.

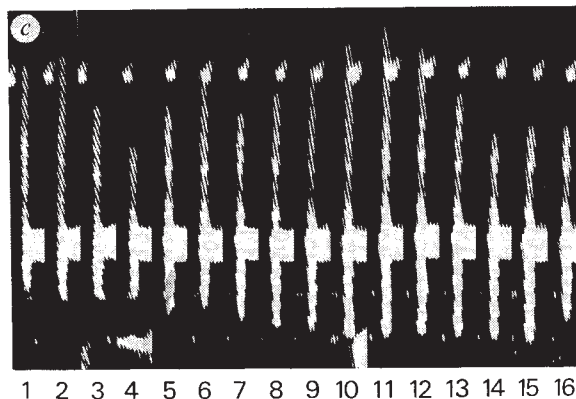
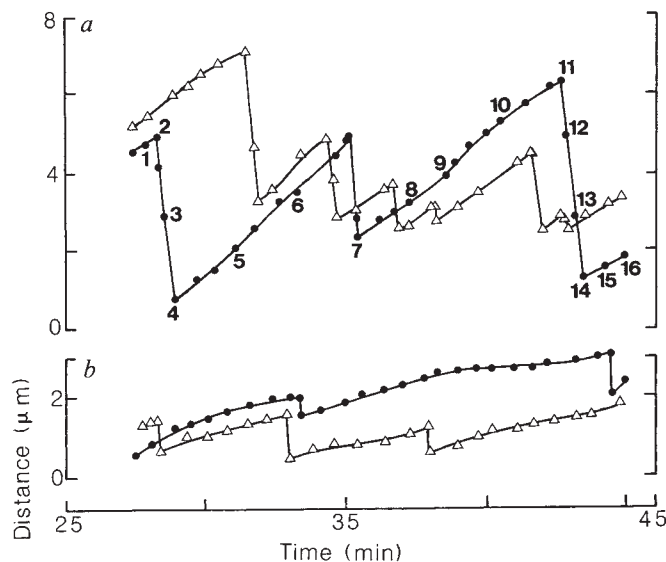


Fig. 2 Interconversions between the growing and shortening phases at ends of a single microtubule. The change in microtubule length at each end was followed by dark-field microscopy and recorded on videotape as described in Fig. 1. The dynamic video images, which were superimposed on the monitor screen of a personal computer (Fujitsu FM 77) with the aid of a superimposer (Fujitsu MB22610), were analysed as a function of time. **a, b,** The distance between one of the ends and an arbitrary fixed point on the microtubule was measured so that increase and decrease in the distance correspond respectively to the growth and shortening of the microtubule. The length change at an active (**a**) or inactive (**b**) end of an individual microtubule is shown. The curves were obtained from the microtubules designated as b (●) or e (△) in Fig. 1. **c,** Successive frames were obtained from the video image of the microtubule designated b in Fig. 1. Scale bar, 5 µm. Each numbered datum point in **a** has a one-to-one correspondence with the successive video frames in **c**. The upper and lower ends of the microtubule shown in **c** correspond to the active and inactive ends, respectively.

did not move. Despite the difference in the manner of attachment, the filaments underwent phase conversions in a similar manner (Fig. 2), indicating that the interaction between a microtubule and a glass surface produces only a small, if any, effect on the nature of the phase conversion.

The frequencies of phase interconversion at the two ends of a microtubule are distinct from each other, being 0.63 and 0.23 min⁻¹. We designated the ends which had higher and lower interconversion frequencies as active and inactive ends, respectively. The active end differs from the inactive end not only in interconversion frequency but also in growth and shortening rates. In the growing phase, the active end elongates about three times faster than the inactive end (Table 1); in the shortening phase, it shortens more slowly than the inactive end. In general, at the active end, a microtubule grows quickly, alternating frequently between the two phases and fluctuating greatly in length, while the reverse is true at the inactive end, where growth is slow, phase alternation infrequent and there is a little fluctuation (Table 1).

It is well established that a microtubule has a structural polarity and that the 'plus' end grows about three times faster

than the 'minus' end^{6,7}. Hence, the active and inactive ends designated in this study must correspond respectively to the plus and minus ends. Despite the independent alternation of phases of the ends which makes the time course of a length change quite unique in each microtubule, similar rates of growth were obtained with active ends and this rate changed very little during the observation period (Fig. 2). This result also holds for the other three kinetic parameters: the shortening rate of the active end, and both the growth and the shortening rates of the inactive end. These homogeneities in rate indicate that each end has a well-defined molecular structure unique to the respective phases and suggests that there is no intermediate phase. The homogeneities also indicate that the concentration of tubulin

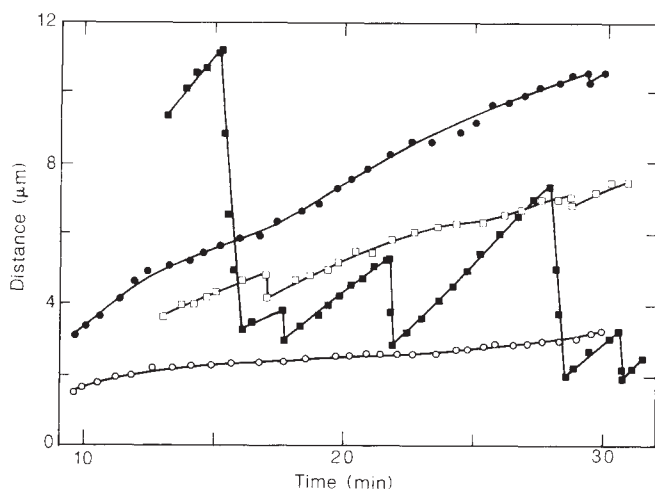


Fig. 3 Effect of MAPs on microtubule instability. Microtubules are formed as described in Fig. 1 except for the presence of MAPs (0.24 mg ml^{-1}). Changes in microtubule length at the active end (●) and the inactive end (○) were measured as described in Fig. 2. In the absence of MAPs, both the active end (■) and the inactive end (□) show the phase conversion in a similar manner to that in Fig. 2. MAPs were purified by phosphocellulose column chromatography as described previously⁸.

subunits is constant during the observation period because a change in subunit concentration would cause a change in growth rate. In other words, we observed the phase conversions in an apparent steady-state condition of microtubule assembly and disassembly (see below).

As shown in Table 1, the shortening rate is much higher than the growth rate at both the active and the inactive ends of the microtubules. Hence, once an end switches to the shortening phase, the microtubules often shorten all the way to disappearance. But in practice this is not the case. The active end stays in the growing phase for a period which averages 10 times longer than it stays in the shortening phase (Table 1). As a result, the microtubule length lost in the shortening phase is compensated for by an elongated length in the growing phase. On average, a microtubule loses $2.4 \mu\text{m}$ during one shortening phase and recovers $1.8 \mu\text{m}$ during a single growing phase. A similar compensation takes place at the inactive end: $0.7 \mu\text{m}$ is lost in one shortening phase and $1.2 \mu\text{m}$ recovered in one growing phase. Therefore, a microtubule does not become so short as to disappear, although its length does fluctuate greatly. This evidence also implies that the net amount of microtubules is almost constant, as it would be in a steady state.

Microtubule filaments are stabilized in interphase cells to maintain the cell shape. How does a microtubule prevent dynamic instability *in vivo*? The purified tubulin used here contained almost no MAPs, whereas in the partially purified tubulin preparation used elsewhere, MAPs make up $\sim 20\%$ of the total protein⁸. Microtubules in the tubulin preparation grow continuously without any shortening phase, and after reaching the steady state they maintain constant length and do not exhibit any dramatic shortening. This suggests that MAPs suppress phase conversion and stabilize microtubules in the growing phase. The results shown in Fig. 3 confirm that MAPs can stabilize both ends of a microtubule as well as their well-known property of nucleation for tubulin polymerization⁸. A detailed description of the effects of MAPs on the stability of microtubules will be published elsewhere.

A GTP cap model has been proposed as the mechanism of phase transition^{2,9,13,14}. When tubulin monomers bound to GTP and ligand are incorporated into a microtubule, the GTP is hydrolysed at a constant rate to form a GDP-tubulin lattice. If the polymerization rate is high enough at an adequate concentration of free tubulin, however, the GTP-tubulin lattice will form

near the ends of the microtubule because the hydrolysis cannot follow the polymerization¹⁰. This lattice, or GTP cap, has been proposed as a mechanism to stabilize the growing phase. On the other hand, when the polymerization slows down with a decrease in tubulin concentration, the GTP cap size is predicted to decrease and the GDP lattice to become exposed on the microtubule end. When this happens, rapid depolymerization of the GDP-tubulin takes place which continuously exposes GDP-tubulins at the end until the microtubule disappears or some rare event recaps the microtubule².

Our results raise some questions about the GTP cap hypothesis as microtubules can interconvert frequently between the two phases without changing polymerization rate. The active end was frequently in the shortening phase at the tubulin concentration used here. Therefore, if the GTP cap hypothesis is correct, the inactive end may always be in the shortening phase because the polymerization rate at the inactive end is only one-third of that at the active end. To clarify the molecular mechanism of the phase conversion, more detailed studies, especially of the effects of GTP, are necessary.

The cytoskeleton and the mitotic spindle can disintegrate rapidly without decreasing the free tubulin concentration in the cell. These structures can also be formed in a range of tubulin concentrations similar to that at which they disintegrate. These cellular events could be related to our demonstration that growing and shortening microtubules really coexist under steady-state conditions.

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Evidence that reserve cells are a source of regenerated adult newt muscle *in vitro*

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Skeletal muscle of adult, metamorphosed urodele amphibians regenerates despite the absence of satellite cells¹, the myogenic reserve cells present beneath the external lamina of muscle fibres in larval urodeles and in other vertebrates^{2–9}. Adult-stage muscles of several urodeles examined (*Notophthalmus viridescens*^{1,10}, *Ambystoma maculatum*¹⁰, *Triturus vulgaris*, *Triturus cristatus*¹¹, *Hynobius tokyoensis*¹² and *Ambystoma mexicanum*¹³) have instead a unique cell type enveloped in its own external lamina. Several studies suggest that these cells are larval satellite cells that have become completely surrounded by a segment of external lamina

during metamorphosis¹⁰⁻¹². Although Popiela¹⁰ termed these cells 'pericytes', he suggested that they might function as a myogenic reserve population. Cherkasova, who was the first to recognize these cells as distinct from vascular pericytes, termed them post-satellite cells and also proposed that they might be myogenic¹¹. Here, we present evidence that post-satellite cells in adult *N. viridescens* give rise to regenerated myotubes *in vitro*. The myotubes could be stained with MF20, a monoclonal antibody to purified skeletal muscle myosin¹⁴, and also with 22/18, a monoclonal antibody that reacts with an antigen present in newt-limb blastema myogenic cells¹⁵. The presence of myogenic post-satellite cells in adult newt muscle provides an alternative to the hypothesis that myoblasts in regenerating adult urodele limbs arise through dedifferentiation of mature myofibres¹⁵⁻¹⁷.

Recent results from our laboratory demonstrate that adult newt muscle undergoes repair regeneration *in vivo* even though the original myonuclei degenerate¹⁸, and that it is able to form new myotubes from muscle explants *in vitro*, where it is apparent that mature fibres do not give rise to myogenic cells by dedifferentiation¹⁹. Those studies supported the idea that adult newt muscle can regenerate from a reserve cell population, whose identity remained unknown.

Post-satellite cells in adult *N. viridescens* limb muscle are similar at the fine structural level to those described in other urodeles, with heterochromatic nuclei and varying amounts of rough endoplasmic reticulum, Golgi and pinocytotic vesicles (Fig. 1). Although post-satellite cells can resemble fibroblasts, their distinctive feature is that each is enveloped in an external lamina. In electron micrographs, we observed only post-satellite cells immediately adjacent to myofibres away from nerves or vascular elements. Light microscopy with serial sections showed that cells in this location usually have basophilic cytoplasm that is sometimes wrapped partially around the adjacent myofibre (Fig. 2b). To test our ability to identify post-satellite cells by their location and cytology at the light microscopic level, 11 such cells in thick Epon sections from both uninjured and cultured muscle were re-mounted and examined at the fine structural level. All 11 possessed intact external laminae, which in the cultured muscle often appeared loosened (Fig. 2a), possibly due to some rounding up of the cells. Therefore, in the subsequent steps of this experiment, post-satellite cells were identified by serial sectioning and light microscopy alone.

We found that post-satellite cells began synthesizing DNA *in vitro* earlier than did any other cell type present in adult newt muscle. Explants of forelimb muscles were prepared according to methods described previously¹⁹ and exposed to ³H-thymidine for 24 h at 2, 4, 6 or 8 days in culture. Incorporation of the label by post-satellite cells and other cell types was determined autoradiographically for each of those days. Up to day 6, ³H-thymidine incorporation was confined to post-satellite cells; no labelled perivascular cells, endothelial cells, perineural cells, Schwann cells or tendon fibroblasts were observed. By day 8, however, an occasional cell of these types was labelled. Of those cells that met our criteria for identification as post-satellite cells by light microscopy, 29.6% (134 of 452 cells in six explants) were labelled with ³H-thymidine on day 6. Only three labelled cells could not be assigned with certainty to a particular histological type because of their appearance and location in the tissue. Thus, restricting the exposure to ³H-thymidine to just the first 6 days in culture appears to provide a means of selectively labelling post-satellite cells, although not all of them incorporate the label during this period (for example, the cells in Figs 1, 2).

Labelled cells identified as post-satellite cells (on the basis of their location and basophilia in serial-section autoradiographs of newt limb muscle exposed to ³H-thymidine continuously for 48 h on days 4 and 5 in culture) were re-mounted for electron microscopy. Some of these cells could be confirmed as post-satellite cells by the presence of intact external laminae (Fig. 3), but many of them lacked intact laminae. As cells lacking intact laminae were not observed in uninjured muscle, we assume that

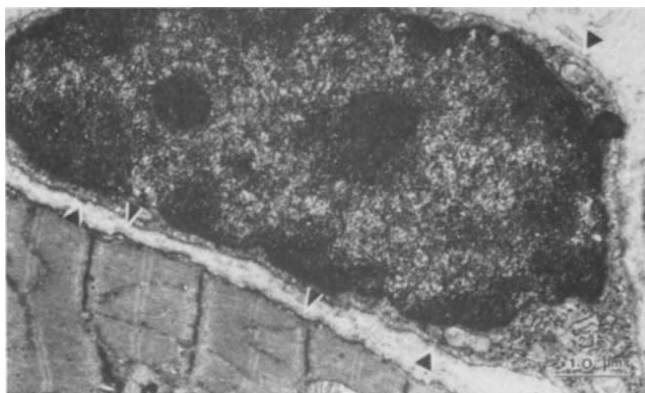


Fig. 1 A post-satellite cell in newt limb muscle after 10 days *in vitro*. Note its external lamina (large arrowheads) and that of the adjacent muscle fibre (small arrowheads). This post-satellite cell did not incorporate tritiated thymidine.

Methods. One μ Ci of ³H-thymidine was added to cultures on day 5. After 24 h the medium was replaced with medium containing 4×10^{-5} M cold thymidine, and this medium was changed at least three times over the next few hours. The cultures were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, after 6 days *in vitro*. Fixed explants were removed from the plates and either embedded in methacrylate or post-fixed with OsO₄ in 0.1 M phosphate buffer at pH 7.4 and embedded in Epon. Serial sections (3 μ m) were coated with NTB2 emulsion (Kodak). After exposure for 1 week at 4°C the autoradiographic preparations were developed, then stained with toluidine blue. Labelled and unlabelled cells in the Epon-embedded muscle tissue were photographed, and selected sections were re-mounted for fine structural analysis.

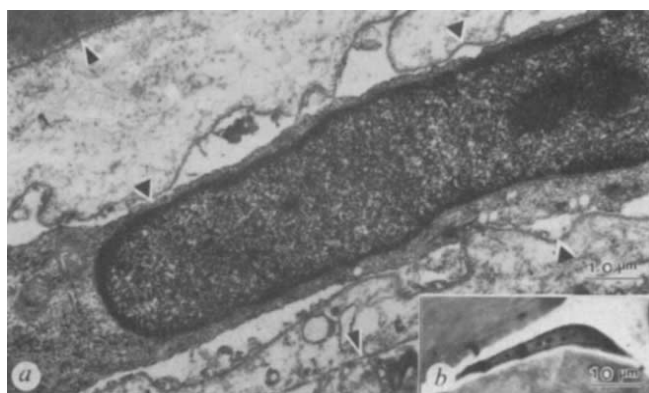


Fig. 2 a, A post-satellite cell in a 10-day explant of newt limb muscle prepared as described in Fig. 1 legend. This cell did not incorporate tritiated thymidine. Note the pinocytotic vesicles along the lower right edge of the post-satellite cell and its loose external lamina (large arrowheads) characteristic of post-satellite cells in injured muscle. Although the two adjacent muscle fibres are degenerating, their external laminae are intact (small arrowheads). b, Light micrograph of this cell prior to re-mounting, demonstrating its location with respect to the two adjacent muscle fibres.

they are post-satellite cells that lost their external laminae before or shortly after synthesizing DNA, although the steps in this process remain to be elucidated.

Cells labelled with ³H-thymidine *in vitro* as described above were followed until they formed regenerated myotubes in longer-term cultures. When muscle explants labelled with ³H-thymidine for 48 h on days 4 and 5 were maintained for periods of up to 4 weeks, myotubes were observed on the culture plates within 14 days *in vitro*. Following autoradiographic processing, many myotubes were seen to contain ³H-thymidine-labelled nuclei (Fig. 4). Immunocytochemical staining with antibody MF20 (ref. 14) confirmed the myogenic nature of these multinucleated



Fig. 3 *a*, A post-satellite cell in a 10-day explant of newt limb muscle labelled with ^3H -thymidine on days 4 and 5 and processed as described in Fig. 1 legend. Note the loose external lamina of the post-satellite cell (arrowheads). *b*, Light micrograph of this cell (*p*) demonstrating its location with respect to an adjacent muscle fibre on one side and a fibroblast (*f*) on the other. Note the silver grains over the nucleus of the post-satellite cell.

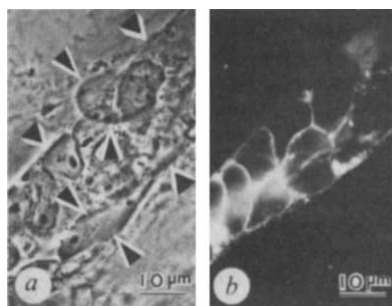


Fig. 5 *a*, A phase-contrast micrograph of a myotube (large arrowheads) in a 21-day culture of newt limb muscle. Two mononucleated cells are also visible (small arrowheads). *b*, The same myotube demonstrating 22/18 immunofluorescence.

Methods. Cultures grown on collagen-coated glass coverslips were fixed in acetone and stained with monoclonal antibody 22/18 (given by Dr Jeremy Brockes) according to the protocol of Kintner and Brockes¹⁵, followed by fluorescein-conjugated rabbit anti-mouse IgG (1:100; Miles). Controls omitting the monoclonal antibody or substituting a known non-reactive primary antibody gave no staining.

cells and in addition allowed some mononucleated cells to be identified as myogenic. Mature fibres within explants were largely degenerated, although they stained weakly with MF20; this is in agreement with our previous conclusion that dedifferentiation of myofibres is not a prerequisite for the regeneration of *N. viridescens* muscle^{18,19}, and the present study points to post-satellite cells as a source of the myogenic cells *in vitro*.

Recently, Kintner and Brockes¹⁵ examined the possibility of myofibre dedifferentiation being involved in regeneration of *N. viridescens* limbs, by using a monoclonal antibody raised against muscle and a second antibody, named 22/18, raised against blastema tissue. In regenerating limbs 14 days after amputation, cells bearing both the myofibre and blastema antigens were found only in the region between the blastema and the intact muscles of the stump. Although those authors observed double-labelled cells also in muscle-forming regions of palette-stage blastemas 28 days after limb amputation, they interpreted the

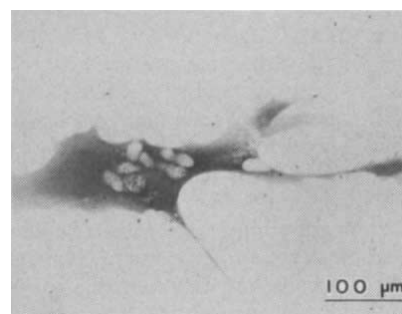


Fig. 4 A myotube in a 14-day culture of newt limb muscle labelled with ^3H -thymidine on days 4 and 5 and stained with MF20 on day 14. Note the silver grains over several of the myonuclei; unlabelled myonuclei are also seen.

Methods. Cultures were fixed in 5% neutral formalin and permeabilized with 0.01% Triton X-100 in 0.02 M phosphate-buffered saline (PBS) for 5 min, followed by 95% ethanol for 5 min. Monoclonal antibody MF20, given by Dr Donald Fischman, was raised against purified myosin heavy chain from adult chicken pectoralis muscle¹⁴. (Immunofluorescent analysis of electrophoretically separated adult newt muscle myosin, performed, with slight modifications, according to the protocol of Bader *et al.*¹⁴, demonstrated the specificity of MF20 for a newt muscle peptide of relative molecular mass (M_r) 220,000 (220K) which migrated close to adult chicken myosin (200K) and purified rabbit myosin (220K).) Supernatant containing MF20 was diluted 1:3 in 0.02 M PBS (0.25 M NaCl) pH 7.6, and applied to the cultures overnight. After three 5-min rinses in the same buffer, the tissue was reacted with 20 µg ml⁻¹ goat anti-mouse IgG (Jackson ImmunoResearch Laboratory) for 8 h in the same buffer, followed by three more rinses with buffer. The MF20 was visualized by incubating cultures in a solution of 4 µg ml⁻¹ mouse peroxidase/anti-peroxidase (Jackson ImmunoResearch Laboratory) in 0.02 M PBS (0.14 M NaCl) plus 1% normal goat serum overnight, followed by three rinses in the same buffer and routine diaminobenzidine treatment. Controls omitting the monoclonal antibody or substituting a known non-reactive primary antibody showed no staining. For autoradiography, culture plates were coated with NTB2 emulsion and processed in the same way as the sectioned material.

appearance of the double-labelled cells in the stump at 14 days as evidence of myofibre dedifferentiation. An alternative explanation for the presence of double-labelled cells in the limb stump at 14 days is that there too it indicates muscle redifferentiation rather than dedifferentiation. Repair myogenesis just proximal to the blastema has been noted at later stages of newt-limb regeneration¹⁷, but we believe it may begin as early as 2 weeks after injury, as it does when newt muscle is injured by mincing *in vivo*¹⁸. To determine whether 22/18 labelling is associated with muscle regeneration *in vitro*, we prepared cultures as before and exposed them to this antibody after new myotubes had formed. The myotubes and some mononucleated cells stained with 22/18 (Fig. 5); taken together with our other results described above, this demonstrates that 22/18 can label myogenic cells apparently derived from post-satellite cells. If post-satellite cells can give rise to myotubes by 14 days *in vitro*, we hypothesize that they do so in limb stumps *in vivo* and that the multinucleated myofibres labelled with 22/18 observed by

Kintner and Brookes in limb stumps 14 days post-amputation were newly repaired or regenerated.

As we have demonstrated the presence of myogenic reserve cells in adult newt muscle, we believe that electron microscope observations of regenerating newt limbs are open to re-interpretation also. Hay¹⁷ showed that mononucleated cells not present in uninjured muscle appear within myofibre external laminae in adult newt limb stump muscle following amputation. Hay postulated that the mononucleated cells arise from the adjacent fibres, enter the blastema and give rise to myoblasts. But neither the origin of these cells nor their possible role in epimorphic limb regeneration has been established rigorously. Even though in the present study we have described repair myogenesis only *in vitro*, we hypothesize that post-satellite cells are also a source of myogenic cells in the epimorphically regenerating limb; this would be consistent with observations that reserve cells are the source of regeneration myoblasts in other vertebrates and would avoid invoking a unique mechanism for the formation of regeneration myoblasts in newts. It may, however, be impossible to test our hypothesis against that of myofibre dedifferentiation until some means becomes available for unambiguously marking either post-satellite cells or the putative viable myofibre fragments.

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Stimulation of oligodendroglial proliferation and maturation by interleukin-2

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There exists considerable evidence that the growth of glial cells can be influenced by T-cell-derived lymphokines and monokines. Astrocytes proliferate in the presence of mitogen- or antigen-stimulated T-cell supernatants¹⁻³, supernatants from human T-lymphotropic virus (HTLV)-transformed T cells³, and purified human interleukin-1 (IL-1; ref. 4). Oligodendrocytes proliferate and differentiate when incubated with supernatants from mitogen-activated or HTLV-transformed T cells³. In addition, we have recently purified a T-cell-derived lymphokine of relative molecular mass 30,000, termed glial growth promoting factor (GGPF), which specifically stimulates the proliferation of oligodendrocytes⁵. The traditional role of interleukins 1 and 2 is in the initiation, propagation and regulation of the immune response⁶. IL-1, released by a variety of cells including monocytes, stimulates T cells to produce IL-2 (ref. 7); IL-2 in turn induces the expansion of T cells that is critical for immune responsiveness. Recently, IL-2 has been shown to induce B-cell proliferation^{8,9} and immunoglobulin secretion¹⁰, indicating that its action is not restricted to T cells. We now report that recombinant human IL-2 influences the growth of glial cells—specifically, the proliferation and differentiation of oligodendrocytes. IL-2 may have a role in the inflammatory neural lesions of multiple sclerosis patients and in the growth of brain glia during injury or disease.

Primary glial cell cultures were established from neonatal rat cerebra by a modification of the McCarthy and DeVellis technique¹¹. Meninges were removed prior to culture. After 7-10 days in primary culture, oligodendrocytes were separated by brief mechanical dislodging. (The overnight shaking technique of McCarthy and DeVellis was not used.) Astrocytes (day 10) were obtained by trypsinization. The two cell populations were

resuspended in culture media containing 10% fetal bovine serum (FBS)⁵. Oligodendrocytes were seeded in Falcon Microtest II plates at a concentration of 2×10^4 cells per well, while astrocytes were seeded at 2×10^3 cells per well. The IL-2-containing material to be tested was serially diluted, and 0.1-ml aliquots were added to the cells. Assays were performed in triplicate. Cells were cultured for various times at 37 °C, pulsed with ³H-thymidine (TdR, 1 µCi per well), and collected after 17 h with an automated cell harvester (PHD harvester, Cambridge Technology). Cells were monitored for purity by immunofluorescence as described previously^{3,5} and by nonspecific esterase staining¹² for contaminating microglia. Oligodendrocyte-enriched cultures were assayed for the presence of the following markers before and after stimulation with IL-2 (see Table 1): galactocerebroside (GalC), a myelin-associated galactosphingolipid that is a surface antigen specific for oligodendrocytes¹³; the A2B5 antigen (which identifies a bipotential glial progenitor cell¹⁴, type II astrocytes¹⁵ and some types of neurones¹⁶); glial fibrillary acidic protein (GFAP), an astrocyte marker¹⁷; tetanus toxin receptor (which identifies neurones¹⁸,

Table 1 Composition of oligodendrocyte-enriched cultures

Marker	Pre-stimulation (%)	Post-stimulation (%)
GalC	86	93
A2B5	12	3
GFAP	5	4
Tetanus toxin receptor	0	0
Nonspecific esterase	5	1

Oligodendrocytes were stained on harvesting (day 7-10), and after a 4-day incubation with recombinant IL-2. Surface staining for GalC was performed for 30 min in the cold with rabbit anti-GalC antibody (1:25), followed by fluorescein isothiocyanate (FITC)-conjugated F(ab')₂ goat anti-rabbit immunoglobulin (1:20; Cappel) for 30 min; and for A2B5 using mouse monoclonal anti-A2B5 (1:20; Sera Labs) for 30 min, followed by FITC-conjugated goat anti-mouse immunoglobulin (1:20; Tago) for 30 min. Surface tetanus toxin receptors were tested for by staining with tetanus toxin, human antisera to tetanus toxin, and FITC-conjugated rabbit anti-human immunoglobulin (1:25; Accurate). Cytoplasmic staining was performed on fixed cells with rabbit antibody to GFAP (1:20; Dako) for 30 min at room temperature, followed by incubation with FITC-conjugated goat anti-rabbit immunoglobulin (1:20; Cappel). Nonspecific esterase staining was performed as described in ref. 12.

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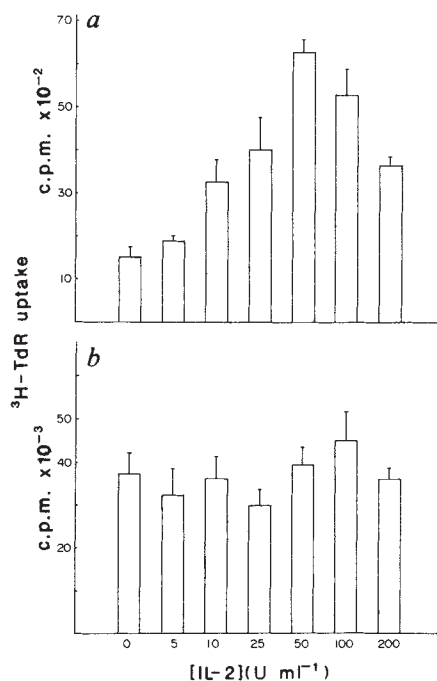


Fig. 1 Effect of recombinant human IL-2 (rIL-2) on oligodendrocyte and astrocyte proliferation. rIL-2 from two sources (Amgen, Thousand Oaks, California and rIL-2 given by Genetics Institute, Boston, Massachusetts) was tested at various concentrations (5–200 U ml⁻¹) on: *a*, oligodendrocytes (2×10^4 cells per well); and *b*, astrocytes (2×10^3 cells per well). The cells were pulsed on day 3 with ³H-TdR, and harvested 17 h later. For oligodendrocytes, three of the experiments were performed with Amgen rIL-2 and three with Genetics Institute rIL-2. The data are expressed as the mean $\pm$ s.d. of these six experiments. For the astrocyte experiments, the values represented are the mean $\pm$ s.d. of three experiments, all performed with Amgen rIL-2. Control media consisted of 50% Dulbecco's modified essential medium (DMEM), 50% Ham's F-12 medium, 2 mM glutamine, 0.1% gentamycin and 10% FBS. Comparable results for oligodendrocyte proliferation were obtained when the experiments were performed in SFM: DMEM (high glucose formula) supplemented with glucose to a total of 6 g l⁻¹, 0.1 mM non-essential amino-acid mixture, 5 μ g ml⁻¹ transferrin, 2 mM glutamine and 0.1% gentamycin. Result for experiment using SFM alone = $2,139 \pm 222$ c.p.m., and for SFM plus 100 U ml⁻¹ rIL-2 = $5,494 \pm 204$ c.p.m.

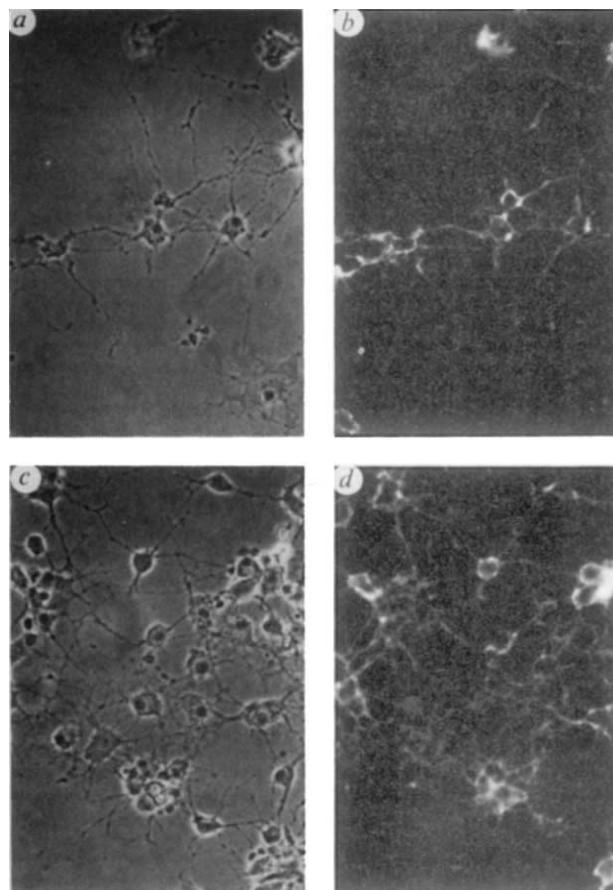


Fig. 2 Photomicrographs of oligodendrocytes after treatment with rIL-2. Phase-contrast microscopy (*a*, *c*) demonstrates the effects of rIL-2 on cultures of rat oligodendrocytes. Cells (2×10^5) were treated for 4 days with either 100 U ml⁻¹ of rIL-2 (*c*, *d*) or control media (*a*, *b*). Immunofluorescence staining for GalC demonstrates that rIL-2 stimulated the growth of oligodendrocytes (*d*). Surface staining for GalC was performed for 30 min in the cold with rabbit anti-GalC antibody (1:25), followed by FITC-conjugated F(ab')₂ goat anti-rabbit immunoglobulin (1:20; Cappel) for 30 min.

glial progenitor cells¹⁹ and type II astrocytes¹⁵); and nonspecific esterase, which identifies microglia¹². Astrocyte populations were more than 95% GFAP-positive before and after stimulation.

We initially tested the ability of partially purified human IL-2 to stimulate enriched populations of both oligodendrocytes and astrocytes. Concentrations of partially purified delectinated IL-2 (Electronucleonics) in the range 0.1–5.0 U ml⁻¹ significantly enhanced the proliferation of both cell types, as measured by ³H-TdR incorporation (data not shown). A different pattern emerged when recombinant human IL-2 (rIL-2) was tested. Two different sources of rIL-2 were tested: 99.5% pure recombinant human IL-2 from Amgen and recombinant human IL-2 from Genetics Institute²⁰. rIL-2 stimulated the proliferation of oligodendroglial cells (Fig. 1*a*), but not of astroglial cells (Fig. 1*b*). Thus, astrocyte proliferation in response to partially pure IL-2 may result from the presence of other factors or contaminants in the IL-2 preparation. A titration curve demonstrates that oligodendrocytes respond to rIL-2 in a dose-dependent manner: responsiveness begins at 10 U ml⁻¹, with maximal proliferation observed at 50–100 U ml⁻¹ (Fig. 1*a*). These rIL-2 concentrations are comparable to those required for human

B-cell proliferation^{8,9}. Figure 2 shows the oligodendrocyte response to rIL-2: 2×10^5 cells were incubated in 24-well Costar plates with or without rIL-2 for 4 days (previously determined to be the optimal time in culture), after which time the cells were counted and assayed for GalC immunofluorescence. Cell counts were determined by trypsinization of triplicate wells, counting the cells per well, and taking a mean of the three values. The results of three separate experiments indicated that cell numbers were increased in cultures containing rIL-2 (Fig. 2*c*; 5.9×10^5 cells), compared with those without rIL-2 (Fig. 2*a*; 2.5×10^5 cells). Over 90% of the cells in both cultures were positive for GalC (Fig. 2*b*, *d*).

Oligodendrocyte proliferation in response to rIL-2 was confirmed by autoradiography^{3,5}. Oligodendrocyte-enriched cultures (89% GalC input cells) were grown for 4 days with or without rIL-2 (100 U ml⁻¹) on sterile coverslips in Costar 24-well plates. The cells were labelled with ³H-TdR (3 μ Ci per well) for 17 h before collection. After harvesting, the coverslips were washed to remove unincorporated ³H-TdR, and fixed in acetone for 10 s. The slides were then immersed in Ilford K2 nuclear emulsion, exposed for 2 days at 4°C, and developed. Sister cultures were stained for GalC after stimulation, and were 92%

GalC-positive. In the presence of rIL-2, 60–70% of the cells incorporated ^3H -TdR, compared with 10% of cells incubated in control media alone.

At the time of addition of growth factor, oligodendrocyte populations were predominantly GalC-positive. However, there were also 12% A2B5-positive cells, on average (Table 1). The bipotential glial progenitor cell which carries the A2B5 antigen has the ability to differentiate into either an astrocyte or an oligodendrocyte, depending on the culture conditions¹⁴. To exclude the possibility that our results could be explained by rIL-2 stimulating these progenitor cells instead of exerting a direct effect on GalC-positive cells, the following experiments were performed: oligodendrocyte cultures were treated twice with anti-A2B5 antibody (1:50) plus complement at 37 °C for 30 min to remove progenitor cells³. These antibody-treated oligodendrocytes then contained fewer than 4% A2B5⁺ cells; but they retained the ability to respond to rIL-2 as determined by ^3H -TdR incorporation ($1,436 \pm 449$ c.p.m., control media alone; $4,876 \pm 222$ c.p.m., with rIL-2). This result indicates that rIL-2 directly stimulates the proliferation of GalC⁺ oligodendrocytes.

Another parameter of oligodendrocyte growth and maturation is the synthesis of myelin basic protein (MBP)²¹. While most oligodendrocytes express the surface antigen GalC¹³, the simultaneous expression of GalC and MBP implies a more differentiated state²². MBP appears during oligodendrocyte development, and accumulates before the onset of myelination²³. As determined by double immunofluorescence staining, an increased percentage of GalC⁺ MBP⁺ oligodendrocytes ($38 \pm 5\%$) was

growth of glial cells can be influenced by specific T-cell lymphokines. Our results differ from those of Giuliani and Lachman⁴, who found that oligodendrocytes did not respond to rIL-2. This discrepancy may be the result of different culture conditions, isolation procedures and age at which the cells were tested. Giuliani and Lachman used mixed glial cultures which consisted of both astrocytes and oligodendrocytes. These cells were cultured for 2 days, at which time rIL-2 was added for an additional 3 days. No information was provided as to the number of GalC⁺ cells or A2B5⁺ cells present before and after stimulation. In the present study, we used enriched populations of oligodendrocytes obtained after 7–10 days in primary culture. The cells which responded to rIL-2 were predominantly GalC⁺ oligodendrocytes.

A previous study by Merrill *et al.*³ indicated that purified human IL-2 stimulated neither astrocytes nor oligodendrocytes. The concentration of IL-2 used in that study was $<10 \text{ U ml}^{-1}$, and we now know that oligodendrocyte responsiveness begins at 10 U ml^{-1} , with optimal proliferation at $50\text{--}100 \text{ U ml}^{-1}$ (Fig. 1a). Thus, the concentration of IL-2 used in the previous study was too low to induce oligodendrocyte proliferation.

In the demyelinating disease of multiple sclerosis (MS), there is an astrocytic reaction consisting of hyperplasia and gliosis which results in the formation of brain plaques^{24,25}. IL-1 is released in the brain following injury⁴, and may mediate inflammation associated with brain injury. In addition, its proliferative effect on astrocytes may promote scar formation in damaged or diseased brain (for example, in MS). The myelin-producing cells, the oligodendrocytes, are to a large extent destroyed during the MS disease process, although a small number do persist and proliferate at the edge of the plaque²⁶. The characteristic inflammatory infiltrate in MS consists predominantly of T lymphocytes, with the T-helper/inducer subset being most numerous at the plaque edge, and T-suppressor/cytotoxic cells more abundant within the plaque²⁷. Both of these T-cell subsets secrete IL-2 when stimulated²⁸. *In situ* lymphoid/mononuclear cells in MS brain secrete immunoregulatory molecules such as IL-2, IL-1 and prostaglandin E_2 , which suggests that these cells are activated²⁹. In addition, staining with monoclonal antibody to IL-2 is observed in areas of MS brain containing very few lymphoid cells, suggesting that brain cells themselves may provide an endogenous central nervous system source of IL-2 or of an IL-2-like molecule²⁹. Astrocytes and glioma cells produce an IL-1-like factor^{30,31}, which indicates that glial cells are able to secrete immunoregulatory molecules. Oligodendrocytes *in vivo* may encounter IL-2 in the periphery as a result of damage to the blood-brain barrier³²; alternatively, they may encounter endogenous IL-2 secreted by infiltrating lymphoid cells, or possibly by other brain cells. While we have demonstrated that oligodendrocytes can specifically absorb IL-2 (unpublished observation), it is unknown whether IL-2 acts on oligodendrocytes via the same type of cell-surface receptor as that required for T-cell and B-cell activation. As in the case of IL-1 and insulin, the receptors on different cell types may be distinct. Separate sites on the IL-1 molecule act on fibroblasts and thymocytes³³, while the insulin receptor on rat brain cells is distinct from those on rat liver cells and human lymphocytes³⁴. The finding that rIL-2 enhanced MBP expression (Table 2) is particularly intriguing; this may be the result of either accelerated synthesis of MBP or selective proliferation of MBP⁺ cells. MBP expression in oligodendrocyte cytoplasm precedes myelination¹⁸. Oligodendrocytes, in the presence of IL-2, may be able to accelerate/enhance their ability to myelinate or remyelinate, which would be beneficial in demyelinating diseases.

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Table 2 Immunocytochemical characterization of oligodendrocytes

Total no. of cells counted			% GalC ⁺	% GalC ⁺ , MBP ⁺
Expt 1	–IL-2	200	89	14.5
	+IL-2	241	92	32.0
Expt 2	–IL-2	214	89	11.7
	+IL-2	228	91	42.5
Expt 3	–IL-2	170	85	8.0
	+IL-2	168	89	40.5

Double immunofluorescence staining was performed on cultures of oligodendrocytes incubated either in the presence of rIL-2 (100 U ml^{-1}) or in control media alone (–IL-2) for 4 days. Oligodendrocytes (2×10^5) were incubated in Costar 24-well cluster plates on sterile coverslips with or without rIL-2 for 4 days, after which cells were stained for surface (GalC) and cytoplasmic (MBP) markers using indirect immunofluorescence. Surface staining was performed for 30 min in the cold with rabbit anti-GalC (1:25), followed by rhodamine-conjugated F(ab')₂ goat anti-rabbit immunoglobulin (1:20; Cappel) for 30 min. The cells were fixed in cold acetone for 10 s, then stained with mouse monoclonal anti-MBP antibody (1:20) for 30 min at room temperature. The cells were then stained with FITC-goat F(ab')₂ anti-mouse immunoglobulin (1:20; Accurate). For each experiment, three separate fields (each with a minimum of 150 cells) were counted by phase-contrast microscopy. GalC⁺ cells were counted using a rhodamine filter, then MBP⁺ cells were determined with a FITC filter. The values shown are the means of the three fields counted for each individual experiment. Identical experiments performed in serum-free medium (SFM) gave similar results: for SFM alone, $9 \pm 1\%$ of cells were GalC⁺, MBP⁺; for SFM plus rIL-2, $38 \pm 2\%$ of cells were GalC⁺, MBP⁺.

detected in cultures containing rIL-2 for 4 days compared with those in control media alone ($11 \pm 3\%$) (Table 2). This finding suggests that rIL-2, in addition to inducing proliferation of oligodendrocytes, enhances and/or accelerates maturation.

Our results demonstrate that rIL-2, a T-cell-derived lymphokine which has previously been shown to influence the growth of T and B cells, is also capable of inducing the proliferation and maturation of rat oligodendrocytes *in vitro*. To our knowledge, this is the first observation of rIL-2 stimulating a non-lymphoid cell, and provides further evidence that the

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Mechanism of membrane damage mediated by human eosinophil cationic protein

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Recent evidence suggests a role for eosinophil granule proteins in contact-dependent antibody-mediated cytotoxicity¹⁻³. Cytolysis may involve a secretory phenomenon whereby granule proteins are released at the site of contact between eosinophil and target cells¹⁻⁴. Several basic proteins have been isolated from eosinophil granules, including the major basic protein⁵⁻⁷, eosinophil cationic protein^{8,9}, eosinophil protein-X¹⁰ and eosinophil peroxidase¹¹. One of the major granule proteins of human eosinophils is the eosinophil cationic protein (ECP)^{3,8,9} which has been shown to damage schistosomula of *Schistosoma mansoni* at concentrations as low as 10⁻⁷ M (Ref. 12). Here, we describe the formation of functional channels by purified human ECP. The transmembrane pores formed by ECP are relatively voltage-insensitive and non-ion-selective, suggesting a role for channel formation by ECP in target cell damage mediated by eosinophils. Channel formation by granule proteins of immune effector cells¹³⁻²⁰ may represent a general and effective mechanism of target cell killing.

ECP was purified from human eosinophil granules by means of a low-pH extraction scheme^{8,9}. The purity of the ECP was

confirmed by SDS-polyacrylamide gel electrophoresis (Fig. 1a) and agarose gel electrophoresis (not shown). Purified ECP showed potent haemolytic activity at submicromolar levels (Fig. 1b). Haemolysis by ECP was not dependent on, or enhanced by, the presence of Ca²⁺, Mg²⁺ or Zn²⁺ but was closely dependent on temperature. Maximal haemolytic activity was observed at 37 °C; below 28 °C haemolysis was decreased several-fold and at 4 °C virtually no haemolysis was observed.

The activity of ECP was also studied using nucleated cells as targets and by monitoring membrane potential changes as a result of incubation with ECP (Fig. 1c). All the cells tested (J774 macrophages, CTLL-A11, P388, chicken embryo myotubes) showed rapid and sustained membrane depolarization.

The permeability increase produced by ECP was not restricted to cell membranes. A lipid vesicle-permeability assay, described previously^{21,22}, was used to study ion fluxes induced by ECP across lipid vesicles (Fig. 1d). Vesicles treated with ECP became leaky to monovalent ions (Fig. 1d) and also showed some permeability to divalent ions (Ca²⁺, Mg²⁺), ¹⁴C-sucrose and the fluorescent markers lucifer yellow and carboxyfluorescein.

Voltage-clamped phospholipid planar bilayers^{23,24} were used to dissect the permeability properties of ECP. Because of the high impedance of these bilayers, small conductance changes can be measured with better resolution than with other techniques. Planar bilayers formed from egg soybean phospholipids were exposed to nucleus-free homogenates of human eosinophils, derived from three hypereosinophilic patients by means of density gradient centrifugation²⁵. Current steps of heterogeneous sizes were immediately observed in the recording, consistent with the incorporation of ion channels into the bilayer (Fig. 2a).

Granules from these eosinophils were isolated by centrifugation through a Percoll density gradient²⁶ and a homogeneous population of granules was obtained, as visualized by electron microscopy (data not shown). Granules were then solubilized in detergent and added to planar bilayers. A significant enhancement of channel activity was observed compared with that obtained with a similar concentration of whole-cell-lysate proteins (Fig. 2b). The enhancement of channel activity was even higher when purified ECP was added to lipid bilayers at the same protein concentration (Fig. 2c), indicating that ECP is probably responsible for most of the pore-forming activity produced by eosinophil granules. Purified eosinophil protein-X (EP-X) and eosinophil peroxidase produced no channel activity under similar experimental conditions (data not shown).

ECP channels are resistant to closing by high transmembrane potentials. Up to 100 mV, the conductance associated with ECP channels showed little or no relaxation to lower steady-state values in response to voltage pulses (Fig. 3a). This is confirmed by a continuous current-voltage (*I-V*) plot, generated by passing a voltage ramp from -120 to +120 mV (Fig. 3b,c). As shown in Fig. 3b, the current trace became noisy, deviating from linearity only at voltages exceeding 60 mV in either polarity. This region of noise or instability probably represents the closing of individual ECP channels which occurs only at high voltages, as demonstrated with bilayers containing low amounts of ECP channels (Fig. 3c). In such bilayers, the voltage-ramp-induced closing of individual channels can be seen as a quantal leap in the current trace (Fig. 3c). Thus, human ECP forms stable transmembrane pores which are relatively resistant to the effects of the electrical field, resembling the channels formed by polymerized C9 (unpublished observations) and by the pore-forming protein isolated from granules of cytotoxic T lymphocytes and natural killer (NK)-like cells^{16,19,20}.

Single-channel fluctuations were observed only at high transmembrane potentials (Fig. 4). In 0.1 M NaCl ECP channels show unit conductances of 120 ± 7 pS (*n* = 432 events; range 95-150 pS). At least two intermediate conductance states were identified, usually observed when channels flickered between open and closed states at high voltages. ECP channels showed

Fig. 1 *a*, SDS-polyacrylamide gel electrophoresis profile of ECP. 10 μ g ECP was applied under reducing conditions to a 12.6% polyacrylamide gel slab. The three closely spaced bands migrating with relative molecular mass (M_r) 18,000–21,000 showed cross-reactivity on immunoblotting, using monospecific polyclonal and monoclonal antibodies directed against ECP. The purity of ECP was also ascertained by double immunodiffusion, agarose gel electrophoresis and by radioimmunoassays using a library of polyclonal and monoclonal antibodies directed against several granule proteins from eosinophils and neutrophils. *b*, Kinetics of haemolysis produced by human ECP at 37 °C. Sheep red blood cells (SRBC) were washed and resuspended to 10^6 ml $^{-1}$ in phosphate-buffered saline (PBS) containing 1% Vitrogen (Gibco) with 1 mM CaCl $_2$. ECP in PBS was added in small aliquots (1:100–1:1,000 dilution) to the final concentrations indicated and haemolysis assayed continuously by absorbance reading at 700 nm. Haemolysis curves represent absorbance recordings traced over with continuous lines. *c*, Effect of ECP on cell membrane potential of J774 macrophage-like cells plated on coverslips, as assayed by the uptake of 3 H-tetraphenylphosphonium ion (3 H-Ph $_4$ P $^{+}$)^{16,18,34}. ECP in PBS was added to cells at 37 °C to 10^{-6} M for the indicated periods (○), at which time the medium was aspirated and the coverslips rapidly washed by immersing them four times in separate beakers containing ice-cold PBS and 1 mg ml $^{-1}$ bovine serum albumin (BSA). Cells were lysed in 0.5 ml of 0.05% Triton X-100 and 0.25-ml aliquots assayed for radioactivity. Values for 3 H-Ph $_4$ P $^{+}$ trapped in the cells were against background and converted into $\Delta\Psi$ values as previously described²². Control: PBS was added to cells. Data points represent average of four experiments. *d*, Ion fluxes in lipid vesicles. The uptake of 3 H-Ph $_4$ P $^{+}$ into lipid vesicles was quantitated by filtration²². The reaction was initiated by diluting 0.1 ml of lipid vesicles into 0.9 ml of 0.25 M sucrose, 1 ml CaCl $_2$, 10 mM HEPES, pH 7.0, containing 50 μ M 3 H-Ph $_4$ P $^{+}$ and 10^{-6} M ECP (○) or control PBS (●). At intervals, aliquots of the reaction mixture were filtered and washed, and the radioactivity remaining on the filter determined²². Data points represent the average of experiments performed in triplicate. All experiments were done at 37 °C.

Methods. ECP used in this study was derived from either normal human blood buffy coat cells or from patients with hyper-eosinophilia. Leukocytes ($\sim 10^{12}$ cells) were homogenized in 0.34 M sucrose and centrifuged at 800g for 15 min to sediment nuclei and large cell debris. The supernatant was then centrifuged at 10,000g for 15 min. The enriched granule fraction was extracted with 10 vol. of 0.2 M ammonium acetate buffer, pH 4, and centrifuged at 20,000g for 30 min. The supernatant was eluted through Sephadex G-75 with 0.2 M ammonium acetate, pH 4, and ECP-containing fractions concentrated by ultrafiltration on a Diaflow PM-10 membrane. ECP was further purified by ion-exchange chromatography on Bio-Rex 70 (200–400 mesh; Biorad), equilibrated with 0.09 M ammonium acetate, pH 8.4, and 8.7% glycerol and eluted on a 0.09–0.9 M gradient of ammonium acetate, pH 8.4–9.3, and 8.7% glycerol. Cells plated overnight on coverslips were washed three times with low-K $^{+}$ buffer (118 mM KCl, 1.3 mM CaCl $_2$, 0.8 mM MgSO $_4$, 5.5 mM glucose, 20 mM HEPES, 9 mM Na $_2$ CO $_3$, pH 7.4) and incubated with 20 μ M 3 H-Ph $_4$ P $^{+}$ for 30 min at 37 °C. Lipid vesicles were formed from a mixture of phosphatidylethanolamine/phosphatidylcholine/cholesterol at a wt ratio of 3:2:1 and 25 mM β -D-octylglucoside in 0.05 M Na-isethionate, 0.165 M sucrose, 1 mM CaCl $_2$ and 10 mM HEPES, pH 7.0 (buffer A). The mixture was sonicated to clarity and vesicles were formed by a one-step 1:100 dilution with buffer A²¹. Vesicles were sedimented by centrifugation at 100,000g for 90 min and resuspended to 10 mg ml $^{-1}$.

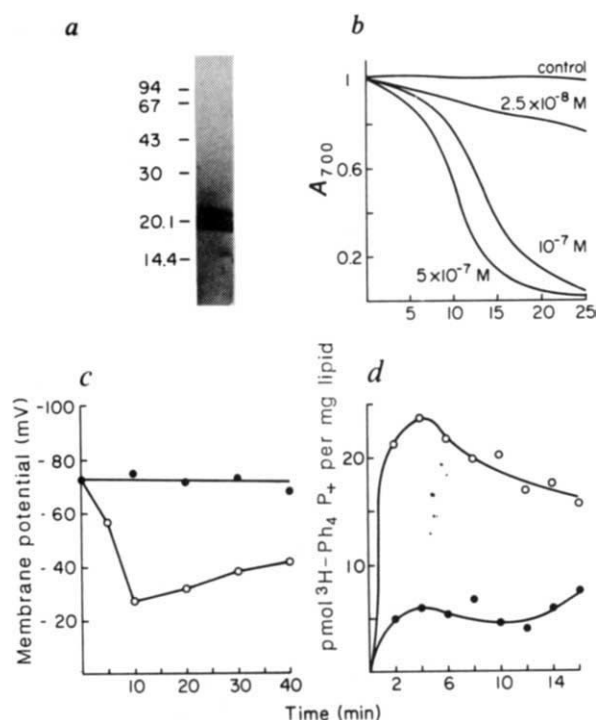
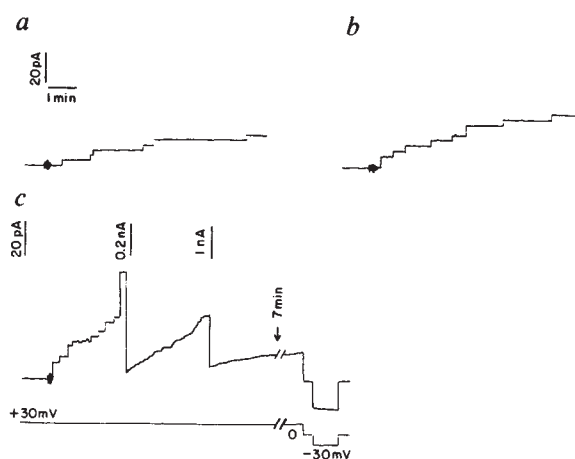


Fig. 2 Effect of eosinophil-derived material and ECP on planar bilayers. Bilayers^{22,24} were formed in 0.1 M NaCl, 1 mM CaCl $_2$, 3 mM NaN $_3$, 10 mM HEPES, pH 7.0 (buffer C), and clamped at +30 mV throughout the experiments unless otherwise indicated (the positive side was named the *cis* side). Baseline conductances did not exceed 10 pS. Reagents were introduced into the aqueous phase of the *cis* side, followed by stirring for 15–20 s (which explains the noise in the recording). *a*, Eosinophils were enriched from the blood of a patient with eosinophilia (42% eosinophil count) by density gradient centrifugation²⁵, resulting in a 94% enrichment, and an aliquot (5 μ g protein, 10- μ l vol.) containing 0.1% Triton X-100 was introduced into the *cis* side of a bilayer bathed in 4 ml of buffer C. Note the upward deflection of current in steps. Two other patients with eosinophilia were used as sources of cells, yielding comparable results. Time resolution 1 ms; temperature 22 °C. *b*, Granules were obtained from eosinophil lysates by centrifugation, and 5 μ g of granule protein in a 10- μ l volume containing 0.1% Triton X-100 was added through the aqueous phase of a bilayer (noise). Same scale as in *a*. *c*, 5 μ g of ECP, purified from granules as described in Fig. 1 legend and dialysed against buffer C, was added to a bilayer. At the points indicated, the current gain was reduced to accommodate the progressive rise of current. The lower trace shows the applied voltage; the parallel bars represent a lapse of 7 min. Note the many-fold enrichment of channel activity compared with *a* and *b*. The voltage polarity was switched to -30 mV to demonstrate that the amplitude of the current remained unchanged, suggesting that channel activity was not dependent on voltage polarity. ECP incorporated into bilayers in the absence of any detergent. The channel activity from granules could also be incorporated without detergent provided that granules were first ruptured by sonication, freeze-thawing or high-salt extraction.



Methods. Eosinophils, enriched as described previously²⁵, were washed in relaxation buffer (130 mM KCl, 4 mM NaCl, 1 mM disodium ATP, 2 mM MgCl $_2$, 10 mM HEPES, pH 6.8) and resuspended to 5×10^7 ml $^{-1}$. Cells were cavitated under N $_2$ (500 p.s.i., 25 min) and collected dropwise in the presence of EGTA, to a final EGTA concentration of 1 mM. After removal of nuclei and unbroken cells (800g, 15 min), the cell extract was sonicated for 1 min on ice and dialysed against buffer C. Triton X-100 (0.5%) was added to this cell lysate and the whole mixture was vortexed vigorously. Cell cavitate in relaxation buffer was centrifuged through a pre-formed continuous Percoll gradient²⁶ (densities 1.02–1.14 g ml $^{-1}$) at 20,000g for 35 min in a Sorvall SS-34 rotor. Granule enrichment was ascertained by β -glucuronidase assay and by ultrastructural examination (not shown). After removal of Percoll²⁶, granules were sedimented at 13,000g for 15 min and resuspended in buffer C to 1 mg protein ml $^{-1}$.

slow kinetics even at high voltages, with the open channel lifetime persisting for up to several minutes (Fig. 4c).

The ion selectivity of ECP channels was determined from the reversal potential necessary to nullify current flow from a 10-fold higher electrolyte concentration (*cis* or positive compartment made more concentrated). The results of such experiments showed permselectivity ratios of 1.00:0.93:0.58:0.21 for K $^{+}$ /Na $^{+}$ /Cl $^{-}$ /Ca $^{2+}$. Similarly, Mg $^{2+}$, Ba $^{2+}$, Zn $^{2+}$, EGTA $^{-}$, HEPES $^{-}$, Tris $^{-}$ and glucosamine (Stokes diameter of 8 Å) also

permeated through these channels. Lipid vesicles exposed to ECP for 16 h at 37 °C were examined by negative-contrast electron microscopy. The vesicle interior was stained with uranyl formate, in contrast to control vesicles which excluded the stain (data not shown). These data indicate that the channels formed by ECP are non-ion-selective and of large functional diameter. The relative molecular mass cutoff associated with these pores is being investigated.

No specific lipid requirement for formation of channels by

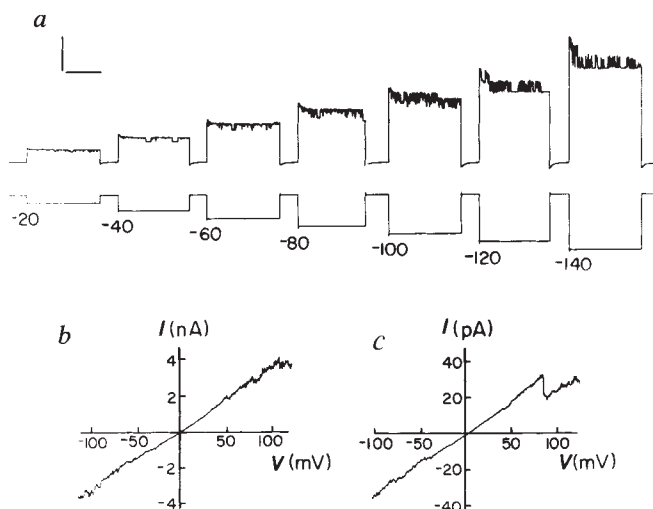


Fig. 3 Effect of electrical field on ECP channels. *a*, A membrane formed in buffer C was treated with 1 μ g of ECP for 30 min prior to application of voltage pulses, in 20-mV increments. Upper and lower traces correspond to current (I) and voltage (V), respectively. Between two pulses, the membrane was allowed to remain at 0 mV for 10 s. Note that the initial capacitive current relaxed to lower steady-state values only at voltages exceeding 80 mV. Increased noise in the recording was associated with current relaxation, corresponding possibly to channel flickering activity at high voltages. Scale bars: 50 pA (vertical) and 20 s (horizontal). *b*, Membrane treated with 1 μ g ECP as in *a* and subjected to a continuous voltage ramp running from -120 to +120 mV in 900 ms. The current record became noisy at high voltage but note the relatively small deviation from linearity even at high voltages. *c*, Membrane containing fewer channels after exposure to 0.1 μ g ECP and subjected to a voltage ramp as in *b*. The downward deflection of current represents closing of one single ECP channel. This result demonstrates that the increase of noise in a many-channel bilayer induced by high voltages (*a*) can be explained by the voltage-dependent behaviour of each individual channel unit.

ECP was observed. Changing the lipid composition of the bilayer from a mixture of phospholipids to pure phosphatidylethanolamine, phosphatidylcholine or cholesterol membranes did not change the overall characteristics of the channel. At 37 $^{\circ}$ C, the rate of channel incorporation into planar bilayers was markedly enhanced (data not shown), an observation which is consistent with the temperature dependence of haemolysis.

Pore formation by ECP may represent the mechanism by which this protein damages target cell membranes. The lack of channel-forming activity associated with other granule proteins such as EP-X or peroxidase suggests that pore formation is a function specifically associated with ECP. Preliminary experiments with MBP do not indicate any functional channel formation associated with this protein. MBP has been shown to damage parasites^{27,28} and cells²⁹ but at concentrations exceeding 10^{-5} M. At these concentrations, it is possible that surface charge interaction with negatively charged membranes may result in

the increased permeability of target membranes^{30,31} without concomitant formation of transmembrane channels.

Pore formation has been associated with a number of different cytolytic proteins in the immune effector system. The prototype of the channel former is the terminal membrane attack complex of complement which appears to be formed mainly by a single protein species, polymerized C9 (ref. 32 and J.D.-E.Y., in preparation). A pore-forming protein has also been isolated from granules of cytotoxic T lymphocytes and NK-like cells¹⁶⁻²⁰, and from the protozoan parasite *Entamoeba histolytica*^{21,33} and implicated in cell killing. Pore formation may therefore represent a general and well conserved immunological mechanism by which effector proteins inflict target membrane damage.

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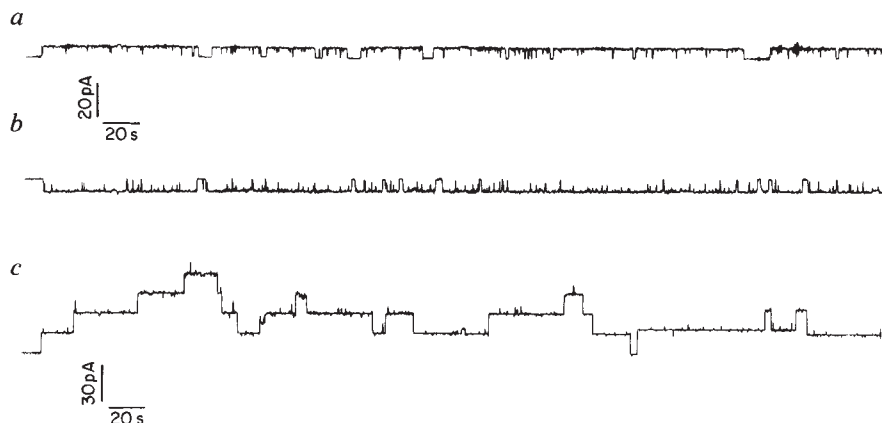


Fig. 4 Single-channel recordings associated with purified ECP. Bilayers, prepared as described in Fig. 2 legend, were treated with 10 ng of ECP. *a* and *b* represent traces from the same bilayer clamped at +50 and -50 mV, respectively. Note the infrequent closing of a single channel (closing represented by downward (*a*) and upward (*b*) deflections) at +50 mV. Same scale for *a* and *b*. *c*, Bilayer treated with 30 ng of ECP and clamped at +120 mV. Note that channel fluctuations are observed more frequently at this voltage.

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A novel cell surface molecule on early B-lineage cells

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B cells and their antibody-secreting progeny represent one of several differentiation pathways that haematopoietic stem cells (HSC) may enter^{1,2}. Cells representing intermediate stages between HSC and B cells have been identified in mammalian haematopoietic tissues^{3,4} and studied intensively over the past decade. This population of early B-lineage cells, termed pre-B, is characterized by cellular proliferation and an orderly cascade of immunoglobulin gene rearrangements⁵⁻⁷, a combination of events leading to the generation of clonally diverse B cells which then migrate to peripheral lymphoid tissues. It remains to be determined what elements determine the polyclonal growth of pre-B cells, how immunoglobulin gene rearrangements are regulated, and what happens to pre-B cells undergoing 'non-productive' immunoglobulin gene rearrangements. These issues could be resolved more easily if early B-lineage cells could be identified precisely and isolated. Here, we describe a cell surface glycoprotein that is selectively expressed by pre-B and newly formed B cells in murine haematopoietic tissues. The molecule, a homodimer formed by disulphide-linked chains of relative molecular mass (M_r) 140,000, is identified by a mouse monoclonal alloantibody called BP-1.

Previous attempts to identify cell surface molecules on pre-B cells have involved immunizing rats with mouse pre-B-cell lines, and fusion of the rat spleen cells to form antibody-producing hybridomas. Monoclonal antibodies which identify cell surface molecules on murine pre-B cells have been produced in this manner, but they do not identify differentiation antigens exclusive to early B-lineage cells⁸⁻¹². We have used an alloimmunization strategy to produce such monoclonal antibodies (named BP antibodies to indicate collaboration between our laboratories in Birmingham and Paris) against pre-B and associated cell types.

Mus spretus, a wild mouse captured in Spain (Mus 3 biochemical group¹³), was selected for immunization, and the Abelson murine leukaemia virus-transformed cell line 18.81 was used as the immunogen; this pre-B-cell line is of BALB/c (Mus 1) origin¹⁴. Following immunization of *Mus spretus* with 18.81 cells, spleen cells were fused with SP2/0 myeloma cells¹⁵. One resultant hybridoma (G10) produced an IgG2a antibody (BP-1) which reacted with 18.81 cells (Fig. 1A) but not BALB/c thymocytes. Immunofluorescence microscopy revealed that crosslinkage of the 18.81 cell surface antigen by the BP-1 antibody (plus a second fluorescein-labelled goat antibody to mouse γ 2a heavy chain) induces the formation of fluorescent caps, suggesting that BP-1 reacts with a surface molecule having a transmembrane connection to intracellular contractile elements.

When normal lymphoid tissues from perinatal mice of Mus 1 strains were examined by indirect immunofluorescence, BP-1⁺ cells were found in the haematopoietic liver and spleen, but not

Table 1 Reactivity of antibodies BP-1 and 14.8 with transformed B-lineage cells

Cell line	Maturation stage	Immunofluorescence intensity*	
		BP-1	14.8
18.81	Pre-B	17.1	20.1
70Z	Pre-B/B	4.0	36.9
38C-13	Immature B	2.9	26.1
4.5	Mature B	1.2	35.5
BCL-1	Mature B	1.0	31.6
WEHI 279	Mature B	1.0	3.2
Ag8.563	Plasmacytoma	1.1	3.3
43.5	Plasmacytoma/pre-B hybridoma	1.0	1.4
56.3	Plasmacytoma/pre-B hybridoma	1.0	1.5

* The ratio of the staining intensities of BP-1 and 14.8 (ref. 5) to the background fluorescence. Methods used for immunofluorescence analyses are described in Fig. 1 legend.

Table 2 Genetic distribution of BP-1 alloantigen expression

Biochemical group	Strain	Geographical origin	Expression of BP-1 antigen
Mus 1	ULA	Palaiseau, France	+
	WLA	Issus, France	+
	DFL	Larzac, France	+
	BNC	Cairo, Egypt	+
	BFM/2	Montpellier, France	+
	38CH	Chiarello, Italy	+
	WMP	Monastir, Tunisia	+
	WGQ	France	+
	Mus 2A	Prague Zoo, Czechoslovakia	—
Mus 2A	MBT	General Toshevo, Bulgaria	—
	MPW	Varsovia, Poland	—
	MBK	Kranero, Bulgaria	—
Mus 2B	MOL	Japan	—
Mus 3	SPE	Santa Fe, Spain	—
	SEI	Ibiza, Spain	—
Mus 4A	XBS	South Bulgaria	—
Mus 4B	ZBN	North Bulgaria	—
	ZRU	Ukraine, USSR	—
Caroli	KAR	Thailand	—

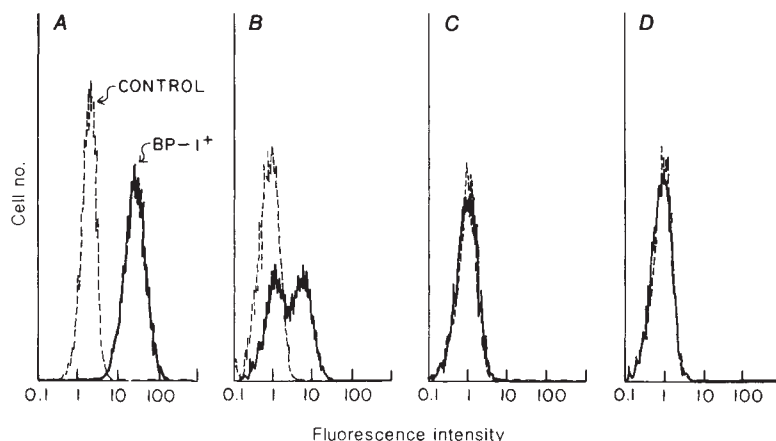
* Unusual immunofluorescence reactivity pattern characterized by the absence of the discrete micro-immunofluorescence patches noted (by fluorescence microscopy) on bone marrow cells from the other Mus 1 strains.

in the thymus. In adult mice, approximately 10% of nucleated cells in bone marrow expressed BP-1 antigen on their surface, but not in the cytoplasm. When bone marrow cells were fixed following surface immunofluorescence staining with BP-1 (fluorescein label)¹⁶ and then stained with anti- μ -chain antibodies (rhodamine label), all cytoplasmic μ^+ cells were BP-1⁺. The intensity of surface staining of BP-1⁺ cells from normal haematopoietic tissues was much lower than that of pre-B cells of the 18.81 line (Fig. 1).

Most IgM⁺ B cells in haematopoietic tissues expressed the BP-1-reactive determinant. In adult bone marrow an average of 61% (s.e. = $\pm 4.6\%$) of the B cells expressed the BP-1 antigen, as did $\sim 80\%$ of B cells in the neonatal liver. In contrast, BP-1⁺ cells were not found in non-haematopoietic lymphoid tissues: thymus, spleen, lymph nodes, intestinal Peyer's patches and peritoneal cells from adult mice. None of the cells expressing the Thy-1 (ref. 17), Mac-1 (ref. 18) or BP-2 (an alloantigen on granulocytes; our unpublished observations) antigen markers of T cells and myeloid cells were BP-1⁺, nor were cells of erythroid, megakaryocytic, endothelial, epithelial or connective tissue lineages.

Fig. 1 Immunofluorescence analysis of the reactivity of various cells with the BP-1 monoclonal antibody. The results obtained with cells from different sources (A, 18.81 cells; B, bone marrow; C, spleen; D, lymph node) are presented as fluorescence histograms with the relative number of cells plotted on a linear scale and the relative fluorescence intensity on a logarithmic scale, both in arbitrary units. Solid lines indicate the staining intensity of the cell-bound BP-1 antibody (IgG2a) detected by fluorescein isothiocyanate-labelled antibodies to mouse IgG2a. Broken lines indicate staining of the cells with the second antibody alone.

Methods. Viable cells, teased from their tissue sources, were suspended in phosphate-buffered saline (PBS, pH 7.4) containing 3% bovine serum albumin and 0.2% sodium azide, filtered through a loose glass wool column, washed by centrifugation and incubated (20 min at 4 °C) with supernatant of hybridoma clone G10-All containing the BP-1 antibody. After extensive washing the cells were incubated with affinity-purified, fluorescein-conjugated goat antibodies to mouse γ 2a heavy chains (0.025 mg ml⁻¹; fluorescein/protein molar ratio = 3.9; Southern Biotechnology Associates). The relative fluorescence intensities of individual cells were measured by automated flow cytometry using the FACS IV instrument (Becton Dickinson Immunocytometry Systems) equipped with a 2-W argon laser. Forward-angle light scatter was used to exclude erythrocytes, dead cells and debris from the analysis. In addition, the bone marrow sample was pre-sorted to remove myeloid cells reactive with the BP-2 monoclonal antibody.



BP-1⁺ and BP-1⁻ populations of bone marrow cells were separated by fluorescence-activated cell sorting (FACS). Because myeloid cells display considerable autofluorescence and the staining intensity of BP-1⁺ cells is relatively low, cells of granulocytic lineage were first removed, on the basis of their immunofluorescence staining with the BP-2 monoclonal allo-antibody. Residual bone marrow cells were predominantly lymphoid in appearance, and about half expressed μ -chains. Analysis of this population by automated flow cytometry revealed a roughly equal division of BP-1-positive and -negative cells (Fig. 1B). When these two subpopulations were separated, ~90% of the BP-1⁺ population were found to express μ -chains while <10% of the BP-1⁻ population were positive for μ -chains. Pre-B cells and most of their B-cell progeny in normal haematopoietic tissues can thus be isolated on the basis of their selective expression of the BP-1 antigen.

Various T-cell lines (BW5147, EL4, B6, KGV, PP16, PP17), macrophage lines (A3, P388D) and a mastocytoma cell line (P815) did not react with the BP-1 antibody. Neoplastic representatives of mature B and plasmacytoid cells also did not react with the BP-1 antibody, while transformed cells of pre-B and immature B phenotypes expressed the BP-1 antigen, the relative amounts of which decreased as a function of maturational stage (Table 1). For example, the BP-1 immunofluorescence staining intensity of the 38C-13 B-cell line, which expresses IgM only¹⁹, was approximately three times the background fluorescence level, whereas the IgM⁺/IgD⁺ BCL-1 line²⁰ has no demonstrable BP-1 reactivity. As is the case for normal B-lineage cells, neoplastic B cells exhibited discordant expression of the BP-1 and 14.8 (B220) antigens.

Control of BP-1 expression was examined by immunofluorescence analysis of hybridomas formed by fusion of the non-immunoglobulin producer Ag8.653 plasmacytoma cells with normal pre-B cells from fetal liver. While these hybridomas display the pre-B-cell characteristics of intracytoplasmic heavy-chain expression and non-rearranged light-chain genes²¹⁻²³, there was no apparent expression of the BP-1 antigen. Three additional hybridomas formed by fusion of Ag8.653 with the 18.81 pre-B-cell line²⁴ also failed to express the BP-1 antigen while continuing to express the 14.8 determinant. This suggests a *trans* gene control of down-regulation of BP-1 expression with progression of B-cell differentiation.

Wild mice belonging to the genus *Mus* have been assigned to five biochemical groups based on protein electrophoretic polymorphisms¹³. Our examination of bone marrow samples revealed BP-1-reactive cells in *Mus* 1 strains, but not in mice representative of other biochemical groups (Table 2). Common

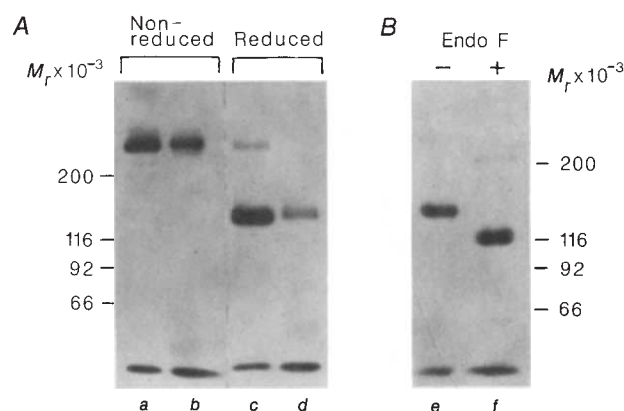


Fig. 2 SDS-polyacrylamide gel electrophoresis (PAGE) of ¹²⁵I-labelled membrane proteins of 18.81 and bone marrow cells. **A**, The BP-1-reactive molecules in lanes *a* and *c* were derived from 18.81 cells, and those in lanes *b* and *d* from normal bone marrow cells. No bands were seen in control lanes, which consisted of material dissociated from wells coated with irrelevant antibody. **B**, BP-1-reactive molecules examined under reducing conditions and following cleavage of complex sugars by overnight treatment with endoglycosidase F at 37 °C (ref. 29).

Methods. Cells ($5.5\text{--}7.5 \times 10^7$) were surface-labelled with 2 mCi of Na¹²⁵I (Amersham) using the lactoperoxidase-catalysed method²⁵, washed and lysed in Nonidet P-40 (1% in 0.05 M Tris pH 7.5) containing 0.15 M NaCl, 0.02% NaN₃, 50 mM iodoacetamide, 1 mM phenylmethylsulphonyl fluoride, 20 mM ϵ -amino-*n*-caproic acid and 0.01% soybean trypsin inhibitor at 4 °C for 30 min. The cell lysate was centrifuged at 10,000g for 15 min and the supernatant precleared by overnight incubation at 4 °C in a plastic well precoated first with goat antibodies to mouse immunoglobulin, then with a mouse monoclonal antibody to chicken IgM. Precleared cell lysate supernatants were incubated overnight in plastic wells precoated with goat antibody to mouse immunoglobulin and the BP-1 antibody. The wells were washed and BP-1-reactive molecules dissociated from the plate by incubation with 2% SDS without or with 5% 2-mercaptoethanol (reducing conditions) for 1 h at 37 °C. Aliquots of antigens dissociated from the immunocomplexes were examined by SDS-PAGE using 5% polyacrylamide slab gels²⁷. Gels were fixed, dried and autoradiographed on Kodak SB-5 film with an enhancing screen.

laboratory strains of inbred mice (BALB/c, CBA/N, CBA/HeJ, Swiss, DBA/2) also had BP-1⁺ cells in their bone marrow.

When surface proteins on 18.81 cells were radiolabelled²⁵ with ¹²⁵I and the BP-1-reactive molecules isolated²⁶ and analysed by electrophoresis in sizing gels (SDS-polyacrylamide, 5–12%)²⁷,

a band of $M_r \sim 250,000$ (250K) was identified under non-reducing conditions. A single band of $M_r \sim 140K$ was generated under reducing conditions for disulphide bonds (Fig. 2). Internal labelling of 18.81 cells with ^{35}S -methionine, followed by sizing gel analysis of BP-1-reactive material, also revealed a molecule comprising disulphide-linked subunits of $\sim 140K$. The 140K band could be detected when the BP-1 antibody was used to identify reactive molecules after electrophoresis of an 18.81 cell lysate (reducing conditions) and transfer of the separated proteins to a nitrocellulose membrane²⁸, indicating relative stability of the antigenic determinant on individual subunits. When BP-1-reactive molecules on 18.81 cells were isolated, reduced and treated with endoglycosidase F (Endo F) to remove *N*-linked oligosaccharides²⁹, the M_r of the subunits was reduced to $\sim 115K$ (Fig. 2B).

BP-1-reactive molecules of identical size and composition were also found in normal bone marrow (Fig. 2A) and in 18.81, 70 Z and 38C-13 cell lines (data not shown). Immunochemical analysis failed to reveal BP-1-reactive molecules on cells from spleen, brain and kidney or on cell lines representative of later stages in B-cell differentiation.

The restricted expression of the BP-1 alloantigen suggests that it could serve as a cell adhesion molecule for early B-lineage cells, or it could be a receptor for a unique induction signal. Noteworthy in the latter regard is the relatively high expression of BP-1-reactive molecules by transformed as opposed to normal pre-B cells. Whatever the functional role of this surface molecule, the BP-1 antibody provides an incisive probe with which to explore early events in the differentiation of normal and neoplastic B-cell clones. The alloimmunization strategy used to produce this antibody could be useful for identifying a variety of highly conserved molecules involved in cellular differentiation.

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Chloroquine and ammonium chloride prevent terminal glycosylation of immunoglobulins in plasma cells without affecting secretion

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The generation of an acidic pH in intracellular organelles is required for several membrane and protein recycling processes. For instance, the internalization of ligands by receptor-mediated endocytosis is followed by the development of an acidic pH inside endosomes; this allows dissociation of the ligand, which is then transported to the lysosomes, from the receptor, which is recycled to the cell surface¹⁻⁴. There is evidence that part of this recycling process involves the distal region of the Golgi complex, where terminal glycosylation occurs: when the plasma membrane transferrin receptor is desialylated by neuraminidase treatment, it acquires new sialic acid molecules after endocytosis and before cell-surface re-expression⁵. Golgi membranes have been shown to contain a proton pump⁶ and the distal Golgi cisternae appear to have an acidic content⁷. Here, we have studied the effects of chloroquine and ammonium chloride, which raise the pH of acidic intracellular compartments⁸, on the processing and secretion of immunoglobulins by plasma cells. Sialic acid transfer to terminal galactose residues, a reaction known to occur in the distal Golgi shortly before secretion⁹, is completely and rapidly inhibited in the presence of these drugs, without significant modification of the secretion rate. This effect is accompanied by a dilatation of the Golgi cisternae and is not rapidly reversible.

Biosynthetically labelled immunoglobulin μ -chains are secreted in the form of pentameric IgM by mouse plasma cells; when fully reduced and alkylated, they have a retarded mobility on SDS-polyacrylamide gel electrophoresis (PAGE) compared

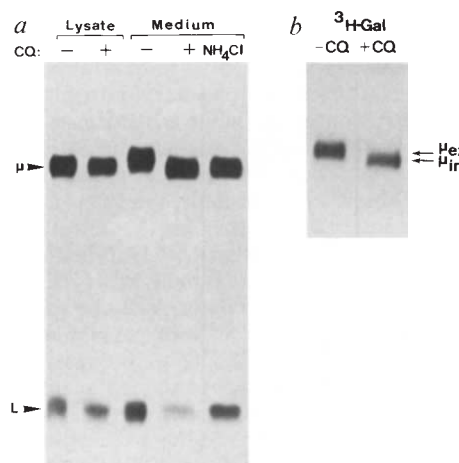


Fig. 1 *a*, Plasma cells, elicited in spleen cell cultures by *Escherichia coli* lipopolysaccharide¹⁸, were labelled biosynthetically with $100 \mu Ci ml^{-1}$ ^{35}S -methionine for 1 h, the lysates and media were prepared and immunoglobulins were immunoprecipitated and analysed by SDS-PAGE¹⁹ (17.5% polyacrylamide). Chloroquine (CQ) was at $100 \mu M$ and ammonium chloride (NH_4Cl) at $10 mM$. μ , μ -chains; L, light chains. *b*, Plasma cells were labelled biosynthetically for 1 h with $100 \mu Ci ml^{-1}$ 3H -galactose in Dulbecco's modified Eagle's medium containing $0.1 mg ml^{-1}$ glucose and 10% dialysed fetal calf serum, in the presence or absence of $100 \mu M$ chloroquine. Secreted μ -chains were on the same gel, analysed as in *a*. μ_{ext} , Mobility of secreted normal μ -chains; μ_{int} , mobility of intracellular incompletely glycosylated μ -chain precursors.

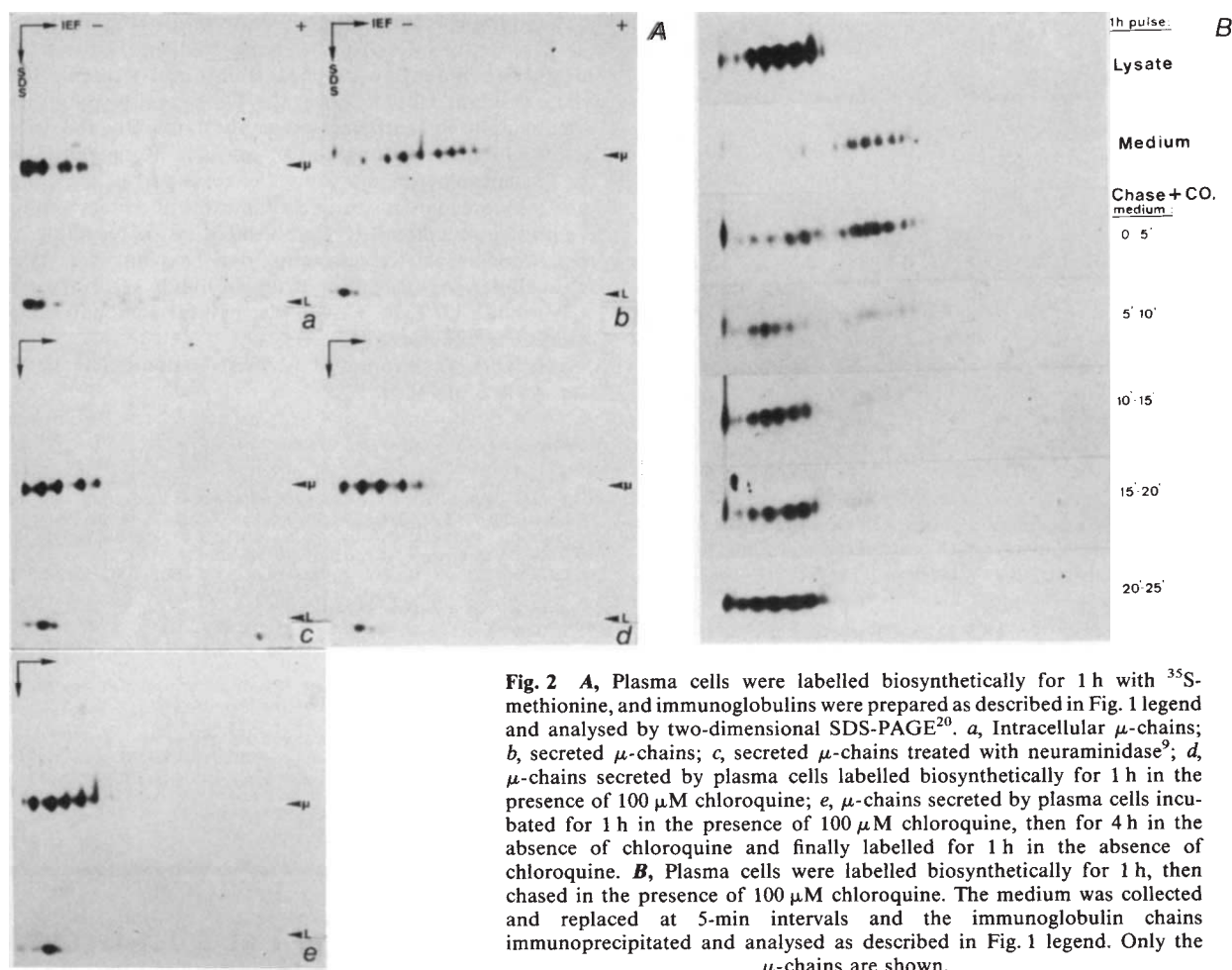


Fig. 2 **A**, Plasma cells were labelled biosynthetically for 1 h with ^{35}S -methionine, and immunoglobulins were prepared as described in Fig. 1 legend and analysed by two-dimensional SDS-PAGE²⁰. **a**, Intracellular μ -chains; **b**, secreted μ -chains; **c**, secreted μ -chains treated with neuraminidase⁹; **d**, μ -chains secreted by plasma cells labelled biosynthetically for 1 h in the presence of 100 μM chloroquine; **e**, μ -chains secreted by plasma cells incubated for 1 h in the presence of 100 μM chloroquine, then for 4 h in the absence of chloroquine and finally labelled for 1 h in the absence of chloroquine. **B**, Plasma cells were labelled biosynthetically for 1 h, then chased in the presence of 100 μM chloroquine. The medium was collected and replaced at 5-min intervals and the immunoglobulin chains immunoprecipitated and analysed as described in Fig. 1 legend. Only the μ -chains are shown.

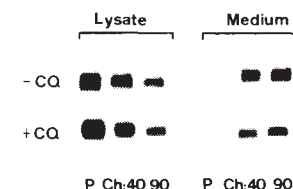
with intracellular μ -chains, due to the complete glycosylation of their oligosaccharide side chains (ref. 9 and Fig. 1). When plasma cells were labelled in the presence of chloroquine or ammonium chloride, the secreted μ -chains had the same mobility as their intracellular precursors (Fig. 1a), indicating that the glycosylation process was impeded. To determine the nature of the missing sugar residues, plasma cells were labelled biosynthetically in the presence of ^3H -galactose, the penultimate sugar residue in complex-type *N*-linked oligosaccharides. Figure 1b shows that the presence of chloroquine did not prevent the addition of ^3H -galactose to μ -chains and that the galactose-labelled chains secreted by the chloroquine-treated cells also had a higher mobility. The degree of sialylation of μ -chains was then assessed by two-dimensional SDS-PAGE (Fig. 2A). Heavy μ -chains secreted by plasma cells have more acidic *p*I's (Fig. 2Ab) than do intracellular μ -chains (Fig. 2Aa) (the vast majority of which have not undergone terminal glycosylation, topologically and chronologically a very late event in the intracellular pathway⁹). This difference in *p*I was totally suppressed after treatment of secreted immunoglobulins with neuraminidase (Fig. 2Ac). Secreted μ -chains synthesized in the presence of chloroquine (Fig. 2Ad) or ammonium chloride (not shown) had the same mobility as unsialylated μ -chains.

Pulse-chase experiments showed that the abolition of immunoglobulin sialylation by these drugs is induced very rapidly, but is not rapidly reversible and has no effect on the kinetics of immunoglobulin secretion. Plasma cells were first labelled biosynthetically for 60 min to allow homogeneous labelling of the immunoglobulins present along the secretory pathway, then placed in a chase medium containing chloroquine; the medium was collected and replaced at 5-min

intervals. Two-dimensional SDS-PAGE showed that μ -chains lacking sialic acid could be detected within 5 min of chloroquine addition, and that sialylated μ -chains were dramatically reduced after 10 min of chloroquine treatment (Fig. 2B). To explore the reversibility of this inhibition, plasma cells were treated for 1 h with chloroquine without labelling, then placed in a medium without chloroquine for 4 h and finally pulse-labelled for 1 h: the secreted μ -chains were still not sialylated (Fig. 2Ae), indicating that the effect of the drug is not rapidly reversible. Similar observations were made with ammonium chloride (not shown). That this inhibition of sialylation has no significant effect on the kinetics of immunoglobulin secretion was found by labelling plasma cells for 30 min then chasing for periods of various length, in the absence or presence of chloroquine; analysis of the μ -chains remaining in the cells or secreted in the medium at these various intervals (Fig. 3) showed that the presence of the drug did not significantly modify the secretion rate.

To explore the possibility that the inhibition of sialylation resulted from a greater sensitivity of sialyltransferase than galactosyltransferase to changes in *pH* or to the presence of high concentrations of chloroquine, plasma cell membranes were

Fig. 3 Plasma cells were labelled biosynthetically with ^{35}S -methionine for 30 min (P), then chased (Ch) for 40 or 90 min, in the presence or absence of 100 μM chloroquine (CQ). The μ -chains present in the lysates of, and secreted by 2×10^6 cells were immunoprecipitated and analysed by SDS-PAGE.



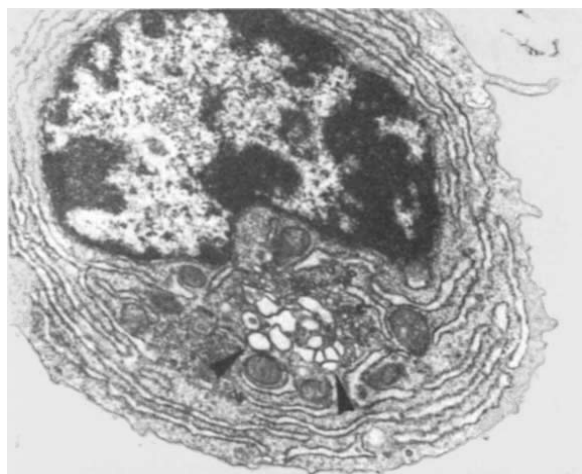


Fig. 4 Electron micrograph of a plasma cell incubated for 1 h in the presence of 10 mM ammonium chloride, showing the dilatation of the Golgi cisternae (arrowheads) ($\times 9,800$).

prepared¹⁰ and their sialyl- and galactosyltransferase activities determined¹¹ at pHs between 5.8 and 7.0, in the presence or absence of 10 mM chloroquine. The activity of neither enzyme was modified within this pH range; chloroquine at this high concentration decreased the sialyltransferase activity by 30–40% and the galactosyltransferase activity by 60%. Thus, defective sialylation does not result from a simple differential effect of pH or chloroquine on the terminal sugar transferase activities.

Plasma cells incubated for 1 h in the presence of chloroquine or ammonium chloride showed two types of ultrastructural alterations: a dilatation of some of the Golgi cisternae (Fig. 4), which were usually positive for thiamine pyrophosphatase activity (not shown), and the presence of very large, clear vacuoles with a scattered distribution, which were observed only in the presence of 100 μ M chloroquine, and which have been described in similar conditions in other cells, such as fibroblasts¹². Golgi alterations were still present 3 h after removal of the drug, while the large vacuoles observed in 100 μ M chloroquine, which are probably swollen lysosomes or endosomes, had disappeared. Thus, only the Golgi dilatation is consistently correlated with the metabolic abnormality.

It is not clear why drugs that raise the pH of acidic intracellular compartments, such as ammonium chloride, chloroquine and monensin, induce an irreversible, probably *trans*, dilatation of the Golgi complex. In the case of monensin, a carboxylic membrane ionophore, the perturbations of the Golgi complex are probably both structurally and functionally more complex since, in addition to an extensive vacuolation of the Golgi cisternae, secretion of immunoglobulins by plasma cells is blocked¹³. In the case of ammonium chloride and chloroquine, it is not obvious why the more limited Golgi alteration is accompanied by inhibition of sialic acid addition but not of galactose addition or immunoglobulin secretion, even though it is usually considered that the two transferases share the same cisternal localization^{11,14–16}. However, in highly secretory cells such as plasma cells, there is an enormous and very rapid vesicular traffic between the distal Golgi cisternae and the cell surface. Sialyltransferase, being the last enzyme to act in the processing of immunoglobulin carbohydrate side chains, may lie still more distally along the secretory pathway; indeed, it has recently been shown in hepatocytes that sialyltransferase is present not only in the Golgi *trans* cisternae, but also more distally in a complex network continuous with these cisternae¹⁷ and in which no galactosyltransferase has been detected¹⁵. If a comparable structure exists in plasma cells, it is conceivable that the enzyme should be constantly carried back towards the distal Golgi cisternae, as part of a membrane protein recycling process. This

may require the constant generation of a pH gradient to allow the persistence of a steady enzyme gradient. Disruption of the pH gradient may allow sialyltransferase and perhaps other membrane proteins (for instance, the Golgi proton pump itself) to remain at the cell surface irreversibly, explaining the persistence of the Golgi alterations and of the lack of sialylation seen in the present experiments. Since the *trans* part of the Golgi complex is involved in the intracellular traffic of endocytosed plasma membrane receptors⁵, its involvement in the recycling of other membrane-associated proteins may explain the effects of intracellular pH-disrupting drugs in highly secretory cells.

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Structure of DNase I at 2.0 Å resolution suggests a mechanism for binding to and cutting DNA

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Bovine pancreatic deoxyribonuclease I (DNase I), an endonuclease that degrades double-stranded DNA in a nonspecific but sequence-dependent manner^{1–4}, has been used as a biochemical tool in various reactions, in particular as a probe for the structure of chromatin and for the helical periodicity of DNA on the nucleosome and in solution^{5–10}. Limited digestion by DNase I, termed DNase I 'footprinting', is routinely used to detect protected regions in DNA-protein complexes¹¹. Recently, we have solved the three-dimensional structure of this glycoprotein (relative molecular mass 30,400) by X-ray structure analysis at 2.5 Å resolution¹² and have subsequently refined it crystallographically at 2.0 Å (ref. 26). Based on the refined structure and the binding of Ca^{2+} -thymidine 3',5'-diphosphate (Ca-pTp) at the active site¹², we propose a mechanism of action and present a model for the interaction of DNase I with double-stranded DNA that involves the binding of an exposed loop region in the minor groove of B-DNA and electrostatic interactions of phosphates from both strands with arginine and lysine residues on either side of this loop. We explain DNase I cleavage patterns in terms of this model and discuss the consequences of the extended DNase I–DNA contact region for the interpretation of DNase I footprinting results.

In our earlier paper¹² we described the binding of Ca-pTp to DNase I the binding of Ca-pTp to DNase I. The di-*p*-nitrophenylester of pTp is a substrate for DNase I (ref. 13). Similar

Fig. 1 Three-dimensional structure and active site of DNase I. *a*, Schematic stereo representation of DNase I with the Ca-pTp binding site indicated. DNase I is a sandwich-type structure with two 6-stranded β -sheets (arrows) forming a large hydrophobic core surrounded by helices (cylinders) and loop regions. The carbohydrate unit (drawn in skeletal form) protrudes by ~ 15 Å from the otherwise compact structure. The disulphide bridges connecting C 170/C 206 and C 98/C 101 are indicated by black dots; the two structurally important Ca^{2+} binding sites, which are distinct from the active-site Ca^{2+} , are marked. *b*, Stereo representation of the C^α backbone of DNase I viewed along the *b*-axis with the Ca-pTp difference density superimposed. The position of the Ca^{2+} ion is clearly defined as the point of highest density in the difference map. Some of the side chains at the active site including His 131 are shown as heavy lines. *c*, Stereo view of the active site of DNase I. Indicated are the position of the Ca^{2+} ion as determined from the Ca-pTp complex and the position of water molecule 390 (black dot) which is in hydrogen-bonding contact with N-3 of the essential H 131 and which, according to the proposed mechanism, acts as the attacking nucleophile. The remarkably strong hydrogen bonds between E 75 and H 131 (2.6 Å) and D 209 and H 249 (2.7 Å) are indicated by dashed lines.

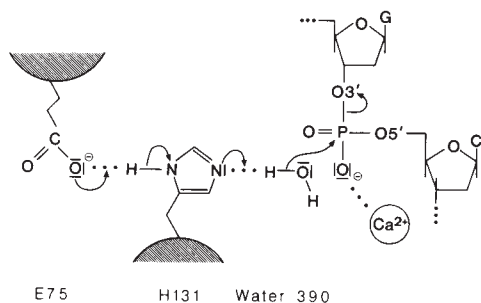
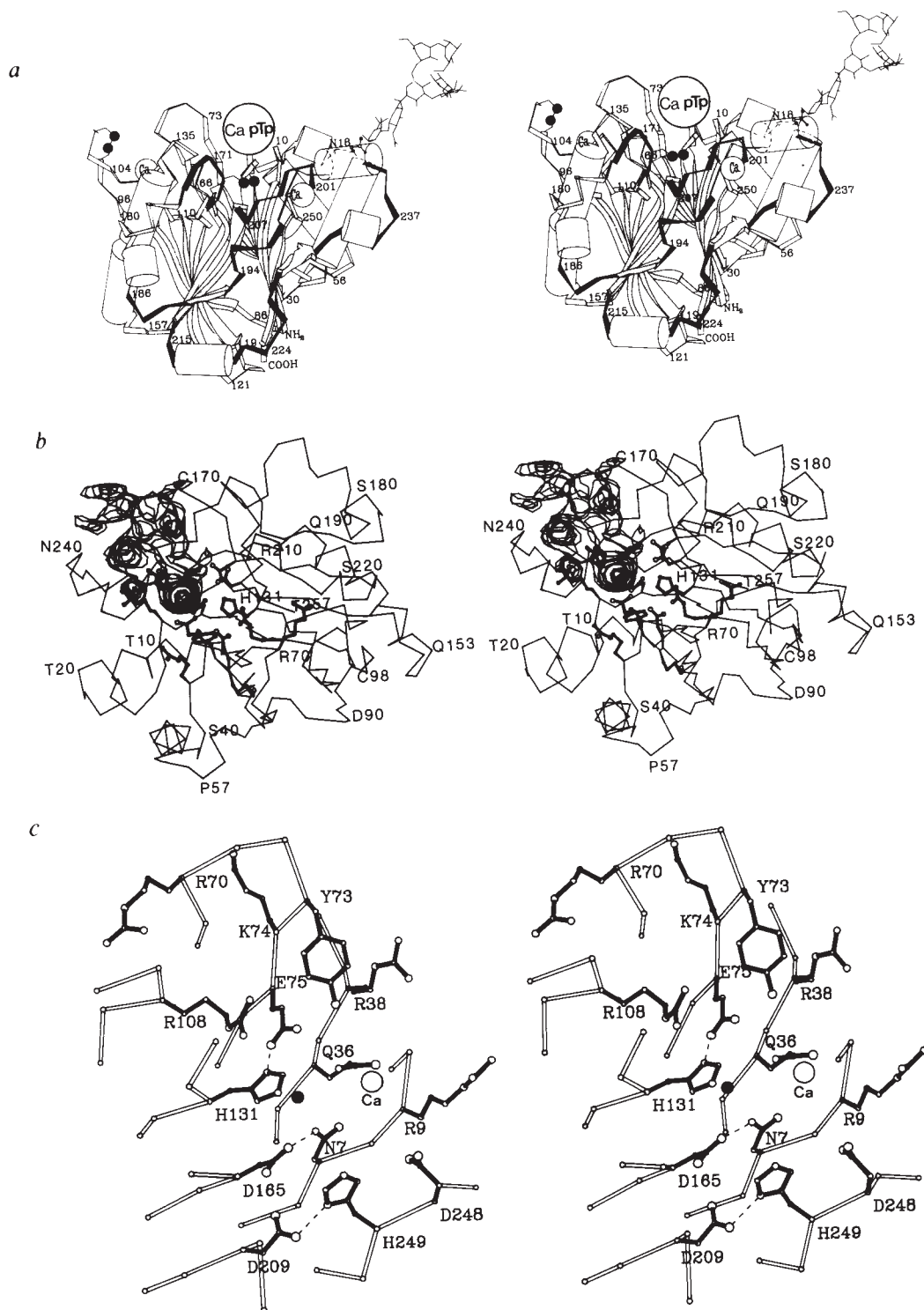


Fig. 2 Proposed mechanism of action. In a general acid-base catalysis, E 75 accepts a proton from H 131, which in turn accepts a proton from a water molecule which then as a nucleophile attacks the phosphorus and cleaves the P-O-3'-bond. The nucleophilic attack of the water hydroxyl is facilitated by the positive charge of the Ca^{2+} ion interacting with the phosphate. This proton acceptor-donor chain Glu-His-water is reminiscent of the catalytic triad Asp-His-Ser found in serine proteases. In agreement with this mechanism, carboxymethylation of H 131 at N-3 as well as protonation by lowering the pH inactivates the enzyme.

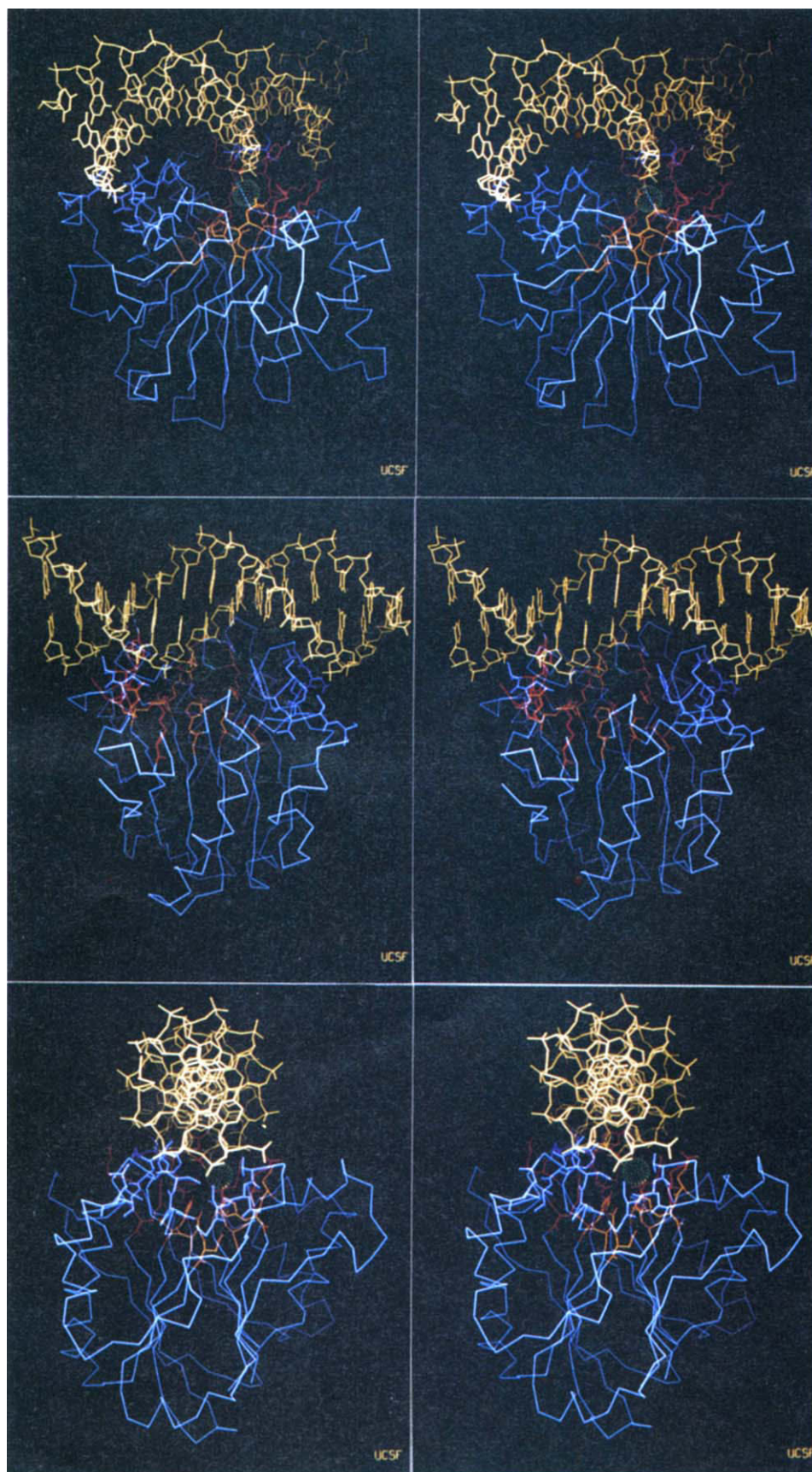


Fig. 3 DNase I-DNA interaction. Three different stereo views are shown, with a piece of B-DNA on top (in yellow) and the DNase I C α -backbone beneath it. The catalytic Ca $^{2+}$ ion, represented by a green dotted sphere, is in contact with the scissile phosphodiester bond (marked in red). Amino-acid side chains directly interacting with the DNA are shown in red and orange, other side chains in the contact region are shown in blue. Y 73, located in the exposed loop R 70-N 71-S 72-Y 73-K 74, is interacting in the minor groove with O-2 of a pyrimidine base. Phosphates from both DNA strands opposing each other in the minor groove make electrostatic interactions with positively charged side chains on either side of the exposed loop (R 9, R 108, R 70 and R 38, K 74). About 5 bp away from the cutting site, additional, mainly van der Waals' type interactions occur in the region of C 170 and T 174. The DNase I contact region extends over approximately one complete turn on one side of the DNA double helix.

to the degradation of DNA, the hydrolysis of this small substrate requires divalent cations and is inhibited by carboxymethylation of the essential residue H 131 (one-letter amino-acid notation). We diffused Ca-pTp into a native crystal and determined its binding site from a 4-Å difference Fourier transform. As expected from the biochemical data, Ca-pTp binds near H 131 at the surface of the protein in a shallow groove between the central β -pleated sheets of DNase I (Fig. 1). (There are two six-stranded β -pleated sheets packed against each other, forming the core of DNase I.) Because of limitations in resolution, the orientation of the nucleotide could not be determined unambiguously but, as we have discussed previously¹², this orientation may not be the same as with DNA. Precisely known from the difference density map, however, is the position of the Ca^{2+} ion bound at the active site in the presence of the nucleotide (see Fig. 1). As described below, we used the position of Ca^{2+} together with the geometry of the active site to derive the model for the DNase I-DNA interaction. The disposition of the Ca^{2+} ion, the amino-acid side chains and the water molecule at the active site is shown in Fig. 1. Note that the assignment of Q 36, which is in contact with the Ca^{2+} ion, has changed compared with our earlier paper¹², because of the insertion of a tripeptide at position 27. (Previously this residue was identified as D 39.) During refinement at 2 Å it became obvious that the chemically determined sequence¹⁴ had to be corrected at several places, the major correction being the insertion of the tripeptide I-V-R at position 27. Remarkably strong hydrogen bonds exist between the carboxylate group of E 75 and the N-1 position of H 131 (2.6 Å) and between D 209 and H 249 (2.7 Å). These salt bridges will shift the pK_a values of the involved histidines to values about one unit higher than expected for a free histidine at the surface of a protein¹⁵. One of the water molecules found in the active-site region (water 390) is within hydrogen-bonding distance of the N-3 position of H 131. As discussed below, this water molecule is the most probable candidate for the attacking nucleophile in DNA hydrolysis.

Mehdi and Gerlt investigated the stereochemical course of the DNase I reaction by nuclear magnetic resonance techniques using substrates with chiral phosphorus atoms¹⁶. Their results show that hydrolysis by DNase I is accompanied by an inversion of configuration at the phosphorus, indicating that a single displacement has taken place during the reaction. The most straightforward explanation for such a reaction course is that the enzyme catalyses the direct nucleophilic attack of a water molecule at the phosphorus atom. A second possibility that was discussed by Mehdi and Gerlt is the formation of an acyl-phosphate intermediate with a carboxylate side chain and subsequent hydrolysis of this intermediate by nucleophilic attack of a water at the carboxyl carbon (and not the phosphorus). This reaction route also implies that there is a single displacement at the phosphorus and would therefore lead to inversion of configuration as well. But for stereochemical reasons, this type of mechanism seems unlikely because even with the most accessible candidate, D 248, the necessary close approach between the phosphate group of the DNA substrate and the carboxylate group appears to be impossible. Based on the geometry of the active site and using in particular the positions of the Ca^{2+} ion in the Ca-pTp complex and of the water molecule 390 in contact with H 131, we propose the following mechanism of action for DNase I (Fig. 2): in a general acid-base catalysis the carboxylate anion of E 75 accepts a proton from H 131, which in turn accepts a proton from the water molecule (water 390), which then, as a nucleophile, attacks the phosphorus cleaving the P-O-3' bond. The Ca^{2+} ion correctly positions the phosphodiester bond with respect to the enzyme and also facilitates the nucleophilic attack of the water hydroxyl through its positive charge. The proton acceptor-donor chain E-H-water of DNase I is reminiscent of the 'catalytic triad' D-H-S found in serine proteases. In agreement with the proposed mechanism, carboxymethylation of H 131 at N-3 (ref. 17) as well as proton-

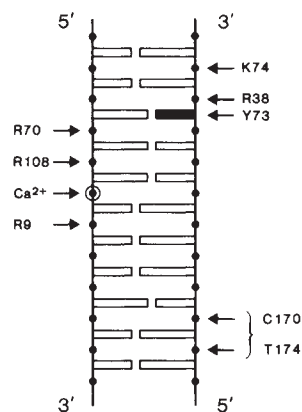


Fig. 4 Schematic drawing of DNase I-DNA interactions. The scissile phosphodiester bond (circle) is anchored by a Ca^{2+} ion to the enzyme. Adjacent phosphates on either side of the cleavage point interact with arginine side chains (R 9, R 70, R 108). Two phosphates of the other strand directly opposite across the minor groove interact with R 38 and K 74. Black rectangle, base interacting with Y 73.

ation of the histidine by lowering the pH (the pH optimum for DNase I lies between 7.5 and 8.5) inactivates the enzyme. In both cases H 131 can no longer act as a general base and accept a proton from the water molecule.

Based on the geometry of the active site and using the crystallographically determined position of Ca^{2+} we have derived a model for the interaction of DNase I with double-stranded DNA in its B-form. Given the constraints imposed by the Ca^{2+} ion, the distribution of the charged residues and the van der Waals' surface of the protein around the active site, the docking was rather straightforward and immediately suggested a starting model similar to the final version shown in Fig. 3. Details of the interaction were elucidated using a colour-graphics display system and the docking program MIDAS (from the University of California at San Francisco). Only the orientations of some exposed side chains of DNase I were altered to optimize the interactions with DNA. The basic features of our model are the rather tight fit of the exposed loop R 70-N 71-S 72-Y 73-K 74 in the minor groove of B-DNA and the electrostatic interactions of phosphates from both DNA strands with positively charged side chains on either side of this loop. The DNA strand being cut is bound at the slightly concave surface between the β -sheets. The scissile phosphodiester bond is anchored to the enzyme through the Ca^{2+} ion, two neighbouring phosphates in the 5'-direction make ionic interactions with R 108 and R 70, the phosphate following in the 3'-direction interacts with R 9 (Fig. 4). Two phosphates of the other DNA strand, directly across the minor groove, are bound to K 74 and R 38. Additionally, mainly van der Waals'-type interactions involving C 170 and T 174 occur 5-6 base pairs (bp) away from the cutting site. The schematic drawing in Fig. 4 shows where the interactions occur on the DNA relative to the point of cleavage. Further ionic interactions, not included in this model, can occur between negatively charged side chains and the phosphate backbone mediated by divalent cations. Possible candidates for this kind of contact are the carboxylate groups of D 248, D 136, E 13 and E 99. The last residue is not visible in the crystal structure because it is located in the flexible loop containing the short disulphide bridge between C 98 and C 101, which, however, is not essential for enzymatic activity¹⁸. Therefore, it appears that any interactions involving this disordered loop are of only minor importance for DNA binding. It is the combination of these interactions—tight fit of a loop region in the minor groove, ionic interactions of phosphates from both DNA strands and van der Waals' interactions somewhat remote from the cutting site—which provides the precise alignment of the DNA-substrate on

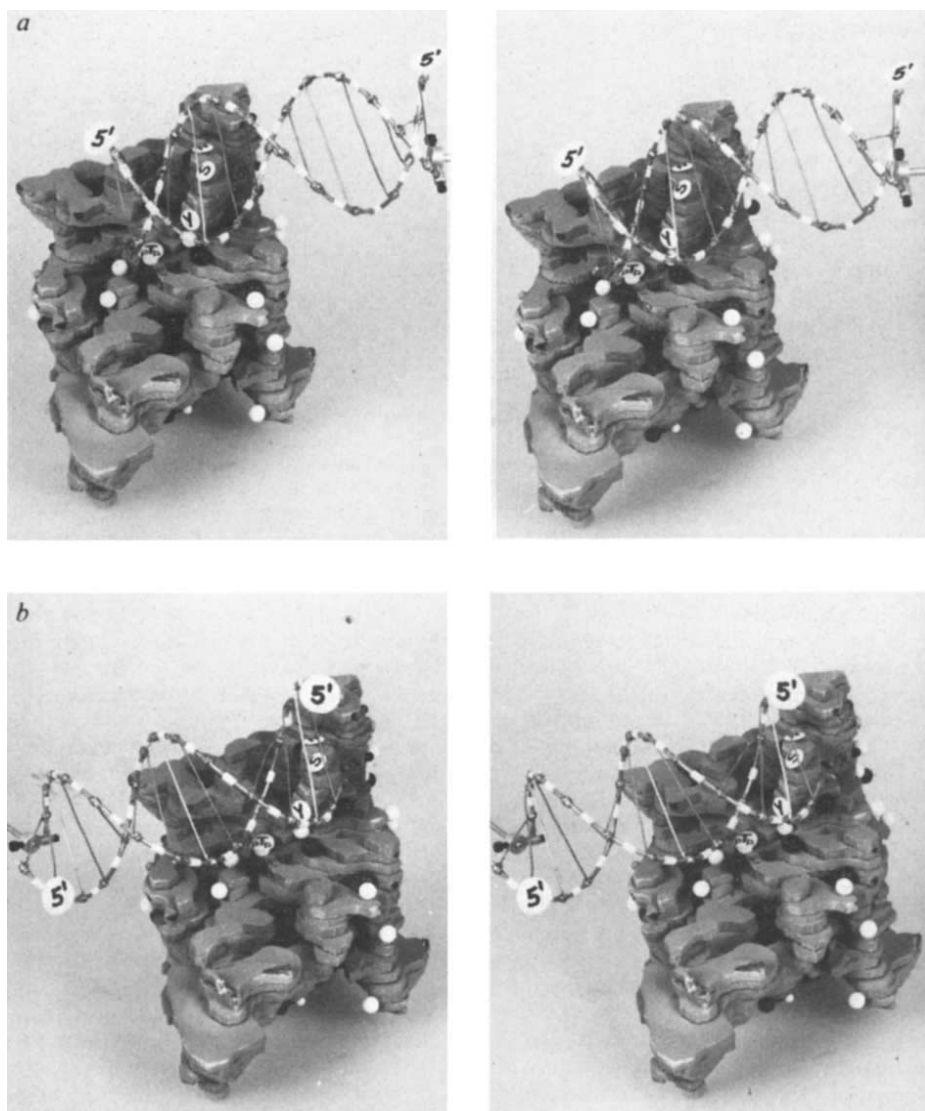


Fig. 5 DNase I cleavage at the 3' end and near the 5' end of DNA. Stereo view of the 5-Å balsawood model of DNase I with a piece of B-DNA at the same scale. Charged residues at the surface of DNase I are indicated by black (R, K) and white (D, E) spheres. Active site marked by pTp, residues N 71, S 72 and Y 73 in the exposed loop interacting in the minor groove of DNA are labelled. *a*, Cutting a bond at the 3' end allows full binding in the minor groove and accurate alignment of the DNA, with R 9, R 108, R 70 interacting with phosphates on the DNA strand to the left of the exposed loop and R 38 and K 74 interacting with phosphates on the other strand. The contacts to R 38 and K 74 to the right of the exposed loop are lost when the cleavage site is 5 bonds away from the 5' end. This feature of the model nicely explains the observation that oligonucleotides are cut near the 3' end or the centre but not, or at very low rates, near the 5' end.

the surface of the enzyme. Y 73, which is located in the exposed loop mentioned above, penetrates into the minor groove of DNA such that its OH-group is in hydrogen-bonding distance from either O-2 of pyrimidines or N-3 of purines. These two atoms are located in equivalent positions relative to the helix axis, so that this interaction in a first approximation will not lead to a purine/pyrimidine discrimination, in agreement with the biochemical results. However, the NH_2 group in position 2 of a G will have a steric effect which could influence the binding (see below).

As shown in Fig. 3, one of the DNA strands follows the direction of the β -sheets and residues at the N- or C-termini of the β -strands or the connecting loop regions participate in the binding or cleavage of DNA. A somewhat similar situation has been found in the *EcoRI*/DNA complex, possibly indicating a general correspondence of β -sheet structure displaying a left-handed twist with a right-handed double helix (J. Rosenberg, personal communication). Whereas the recognition of DNA is different in both cases—major groove interactions in the case of the sequence-specific restriction enzyme, minor groove interaction in the case of the unspecific or better, less-specific DNase I—the catalytic portions of the two enzymes seem to have some resemblance, reflecting the fact that both are endonucleases.

In this context it is interesting to note that DNase I, if present in high concentrations, is able to degrade single-stranded DNA at a rate about four orders of magnitude slower than double

strands¹. The smallest substrate being attacked is a tetranucleotide ($\text{NpN}\downarrow\text{pNpN}$) or a trinucleotide carrying a 3'-phosphate group ($\text{NpN}\downarrow\text{pNp}$), whereas a trinucleotide (NpNpN) is resistant. In good agreement with our binding model, this shows that phosphate groups on either side of the scissile bond are necessary to align the oligonucleotide on the enzyme for cutting (Fig. 4).

Various biochemical results concerning the enzymatic activity and cleavage patterns observed with DNase I can be explained in terms of our model. Evidence for the involvement of Y 73 in the binding comes from a chemical modification study¹⁹. The oligopeptide antibiotic netropsin, which is known from X-ray studies to bind in the minor groove at A+T-rich sequences²⁰, prevents DNase I cleavage at these sites. According to our model, netropsin binding in the minor groove will directly interfere with the binding of the exposed loop R 70 to K 74 of DNase I. Indirect evidence for binding of DNase I across the minor groove stems from a comparison of DNase I cleavage patterns of a hairpin loop structure with a double-stranded piece of DNA of identical sequence²¹.

DNase I digestion studies of self-complementary oligonucleotides show that the enzyme cleaves near the 3' end or the centre of a DNA strand but not, or at very low rates, near the 5' end^{3,21}. The reason for this behaviour is obvious from our model: full binding and accurate orientation of the DNA with respect to the enzyme are only possible with a complete minor groove

allowing for interactions with phosphates from both strands. The situation for binding near the 3' and the 5' ends of a DNA strand are illustrated in Fig. 5. In the case of 3'-end cutting, both strands of the DNA interact with the protein, whereas the contacts to R 38 and K 74 are lost when a phosphodiester bond about 4–5 bonds inwards from the 5' end is cut. One can use this argument to exclude binding across the major groove: exactly the opposite behaviour (cutting all the way to the 5' end and no cutting near the 3' end) would be expected from this kind of model.

Figure 3 shows that the fit of the exposed loop R 70 to K 74 in the minor groove is rather tight and therefore the width of the helix grooves will be a critical factor for the binding. X-ray studies on short double-stranded DNA pieces indicate that the groove width can indeed vary with the sequence^{22,23}. The sequence-dependent variation of the DNase I cleavage pattern and cutting frequencies can therefore be nicely explained in terms of this model by a variation of the minor groove width that directly affects the binding. Among other factors, variation of helix groove width has been discussed by Drew and Travers as a possible explanation for the DNase I cleavage pattern they observed for a 160-bp DNA fragment⁴. They found that the locally averaged probabilities of cleavage were usually higher in regions with random base sequence than in those with runs of A-T or G-C. In agreement with these digestion data, our model predicts optimal binding for a minor groove width of 12–13 Å, a value occurring in 'normal' (that is, random-sequence) B-DNA. There are, however, considerable variations in cutting frequencies of neighbouring phosphates which cannot be explained by a smoothly varying parameter like the groove width. As mentioned above, Y 73 interacts in the minor groove with N-3 or O-2 of the purine or pyrimidine base on the opposite strand, 3 bp away from the cutting site (Figs 3, 4). If there is a G-C (or C-G) pair at this position, steric hindrance between the NH₂ group in the 2-position of the guanine base and the tyrosine hydroxyl group will force the tyrosine into a different orientation compared with the A-T (or T-A) case. Similarly, local changes in the helix twist angle, the base-pair propeller-twist and the roll and tilt angles will have an effect on this interaction. Therefore, it seems probable that the tyrosine interaction in the minor groove has a modulating effect on the cutting frequencies of DNase I.

Another feature of our model is the rather extended binding surface. One side of the DNA double helix is in contact with the DNase I over ~10 bp, that is, over almost a complete turn (see Figs 3, 4). For optimal binding and therefore rapid cutting of DNase I, a piece of DNA of at least this length is required. The well-known fact that the rate of cutting is a function of the length of the DNA fragment^{1,3} can be explained by this feature of the model. The fact that DNase I, unlike a chemical probe, has an extended DNA binding surface has important consequences for the interpretation of DNase I footprinting studies: at least 4 bp to the 5' side and 6 bp to the 3' side of the cutting site are covered by DNase I on one side of the DNA double helix. In other words, DNase I cutting at a given site implies that the protein whose interaction with DNA is being probed by the footprinting experiment is interacting neither in the minor groove following in the 5' direction nor in the major groove following in the 3' direction. Our model nicely explains the difference observed in the size of footprints generated by DNase I and the synthetic DNA cleaving reagent methidiumpropyl-EDTA-Fe(II) (ref. 24). Knowledge of the mode of DNase I-DNA interaction will solve the problem of overestimating the size of DNA binding sites by DNase I footprinting experiments and will therefore significantly increase the accuracy of the method. Another implication of our model is that DNA segments displaying an A- or Z-type conformation will either be resistant to DNase I digestion or will be attacked only after conversion into a B-type conformation. In fact, brominated poly (dG-dC), which was shown by spectroscopic methods to exist as Z-DNA

under the conditions used, was degraded to only 10% by DNase I (ref. 25). Finally, our model can be used to predict the accessibility of phosphodiester bonds by DNase I (angle of attack) in the nucleosome.

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Note added in proof: An X-ray structure analysis of a complex between DNase I and a self-complementary octanucleotide proceeding in our laboratory confirms the proposed binding of the exposed loop R 70 to K 74 in the minor groove of DNA.

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L1 family of repetitive DNA sequences in primates may be derived from a sequence encoding a reverse transcriptase-related protein

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Primate and rodent genomes contain a family of highly repetitive, long interspersed sequences, designated the L1 family or LINE-1 (refs 1–4). Characteristic features of the L1 family sequences such as an A-rich stretch at the 3' end, a truncated 5' end, the existence of significantly long open reading frames (ORFs)^{5–9} and the presence of L1 family transcripts in various types of cells, including pluripotential embryonic cells^{10–15}, suggest that the L1 family is derived from a sequence encoding a protein(s) and dispersed in the genome through an RNA-mediated process. These features of the L1 family are believed to be due to reverse transcription

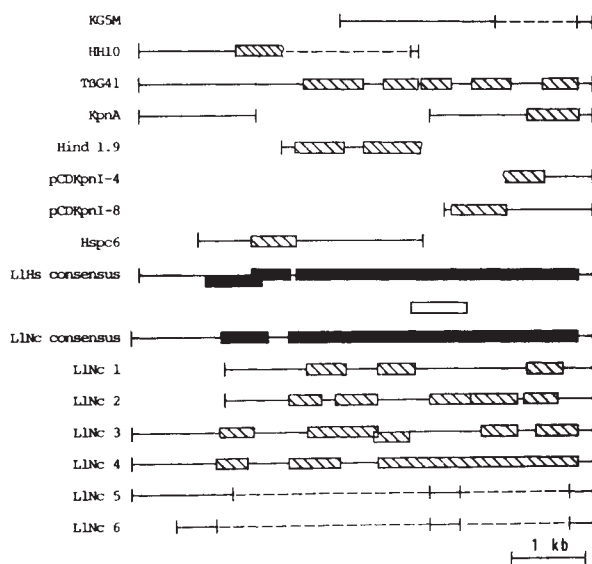


Fig. 1 Schematic representation of the structure of L1 family members and the consensus sequences. The human consensus sequence was deduced from TβG41 (ref. 9), KpnA (ref. 8), Hind1.9 (ref. 5), pCDKpn1-4 and pCDKpn1-8 (ref. 6), HH10 (ref. 25 and K. Yoshioka *et al.*, unpublished), Hspc6 (ref. 26 and K. Yoshioka *et al.*, unpublished) and KG5M (A. Fujita *et al.*, unpublished). The *N. coucang* consensus sequence was deduced from the sequence data of six members (L1Nc1-L1Nc6) analysed here. Filled bars in the consensus sequences and hatched bars in each member show positions of ORFs over 420 base pairs. An open bar between two consensus sequences indicates the region homologous to reverse transcriptase sequences (see text). Broken lines show the region where the sequence has not been determined.

Methods. *N. coucang* DNA, prepared from liver, was partially digested with *Eco*RI, and 15–20-kilobase (kb) fragments were ligated to *Eco*RI-digested arms of bacteriophage vector Charon 4A. After *in vitro* packaging, the recombinant phages were propagated in *Escherichia coli* LE392. The phage library was screened with ³²P-labelled fragments of a human L1 family, TβG41 (refs 9, 27). Positive phage clones were further analysed by the Southern blotting technique²⁸ and selected DNA fragments subcloned into pUC13 or pUC18 (refs 29, 30). Cloned DNA was subjected to sequence determination by the method of Hattori and Sakaki³¹.

beginning at the 3' end of the L1 transcript and terminating prematurely and to the site duplication caused by the insertion of the complementary DNA (reviewed in refs 3, 4). It is likely that this type of transcript is converted to cDNA and inserted into the chromosome through a process similar to that of the formation of processed pseudogenes¹⁶. The above model, however, does not necessarily explain why the L1 family should produce the extraordinarily large number of copies (more than 10⁴ per haploid genome¹⁷) seen during evolution. It seems likely that the progenitor of the L1 family itself carries (or carried) a function which promotes the active dispersion of the L1 family sequence. We reasoned that such a function, if present, must be conserved during evolution and may be shown by comparative analysis of L1 family sequences from evolutionarily distant species. We show here that the L1 family sequence contains an ORF possessing significant sequence homology to several RNA-dependent DNA polymerases of viral and transposable element origins. This provides a plausible explanation for the preferential and active dispersion of the L1 family sequence during evolution.

For a comparative analysis, we chose the human and prosimian, which diverged from each other about 70 million years ago. Six 'full-length' or nearly 'full-length' L1 family members were isolated from a genomic DNA library of slow loris (*Nycticebus coucang*) and their complete (L1Nc1-L1Nc4) or partial (L1Nc5, L1Nc6) nucleotide sequences determined. These members showed a homology of 90% or more among each other

TGNSHITILTLLVNCGLNAPIKRRLANWIKSQDPSVCCIOETHLTCRDLRLKIKGNWNIQANGKQKACVAILVSDK	86
TGSLKGLSIFSNVNCGLNAPIKRRLADWIKQLKPDICCIQSHLTKDKYKRLKVGSSIFQANGKQKAGAILFPADA	101
TDFKPTKIKRDKGHYIMVKGSIQEEELTILNIYAPNTGAPRFIKQVLSDLQRLDSHTIIMGDFNPLSLDRSTROKI	166
IGFKPTKIKRDKGHYIFVKGNTQYDEISIIINIVAPNHNAPOFIRETLTMSNLISSTSVVGDFTPLAVLDRSSKKKL	181
NKDIQELNSALHQADLIDIRYTLNPKSTETTFSSPHHTYSKTDHILGSKTLLSKCKRTEITNCL-SDHSAIKLELRK	245
SKELDLNSTIQLHLLDITRYTFHPNKTETTFSSAGHTYSKIDHILGHSKLSFKFKIETIP-CIFSDHNGIKVELNN	260
KLTONHSTWKLNNLLNDYVHNMKAETKFFETNENKDTTYQLNDWAKAVCRGFIALNAHKRQKERSIDTLISQ	325
RNLHTHTKWLKNNMLKDTWIDEIKKEITKFLQNNQDTHYQLNDWAKAVLGRGFIALQFLKTEREEVNLNGH	340
LKELEKQEQTSKASRQRIETIRAELEKEITKTLQKINESRWFPEKINKIDRLARLKKRREKNOIDTKNDKGI	405
LKLEKEEHSNPFPRKKEITIRAELEKEITKTLQKINESRWFPEKINKIDRLARLKKRREKNOIDTKNDKGI	420
TTDPTETITIREYKYLANKLENLEEMDKFLDTYTLPRNLQEVESLNRPITSSIEAIIINSLPNKKSPPGEGTAEF	485
TTDPTETIKILNEYKYLANKLENLEEMDKFLDTYTLPRNLQEVESLNRPITSSIEAIIINSLPNKKSPPGEGTAEF	500
YQRYKEELVFLKLPQSTKEGILPNSFYEASIIILPKPGDRDTTKENFRPISLNMNADIKLNLKILANOQGHKKLIH	565
YQRYKEELVFLKLPQSTKEGILPNSFYEASIIILPKPGDRDTTKENFRPISLNMNADIKLNLKILNRQGHKKLIH	580
HDQVGFIPAGQWPNIRKSIINIIQHNKRTKDTNHMISDAEAKDFKIQPPMLKPLNKLIDCTYKLIIRAIYDKPTAN	645
HDQVGFIPGSGQWPNIRKSIINIIQHNKRLKNDHMLSIDAEAKDFKIQPPMLKPLNKLIDCTYKLIIRAIYDKPTAN	660
IILNGKLEAFPLKTRGCGPLSPFLFNIVLEVAIAIRQKEIKIQLGKEEVKLSLPADDMIVYLENPIVSAQNLK	725
IILNGKLVKSPFRLTRGCGPLSPFLFNIVLEVAIAIRQKEIKIQLGKEEVKLSLPADDMIVYLENPIVSAQNLK	740
LISNFKSVGKYNVOKSOAFLYTNNTQTESQINSELPPTTASIKRIYLGICLITRVKDLFKENYKPLNEIKEDTNKK	805
VKEYSNVSGKYNVOKSOAFLYTNNTQTESQINSELPPTTASIKRIYLGICLITRVKDLFKENYKPLNEIKEDTNKK	820
NIPCSWGRINIVKMAILPKVIYRFAIPIKLPMTFTELEKTLKFINQKRAHIAKSTLSQNKAGGITLPDFKLKYY	885
NIPCSWGRINIVKMAILPKVIYRFAIPIKLPMTFTELEKTLKFINQKRAHIAKSTLSQNKAGGITLPDFKLKYY	900
ATVTTKAKYQNRDIDOWNTEPSEIMPHIYNYLIDFKPEKNQWQDLSFNKWCWENLAICRKLKDLPLFTYTKIN	965
SVITTKAKYQNRDIDOWNTEPSEIMPHIYNYLIDFKPEKNQWQDLSFNKWCWENLAICRKLKDLPLFTYTKIN	980
SRWIKDLNVRPKTIKLEKNLGTIQDGMGRDPMFKPMATKVKIDRDLKLSFCTAKETTRIVNRQPTXKWKIF	1045
SRWIKDLNVRPKTIKLEKNLGTIQDGMGRDPMFKPMATKVKIDRDLKLSFCTAKETTRIVNRQPTXKWKIF	1060
AIYSSDGLISRIYNELQIVKKTNNPIKKWDRNRHPSKEDIYAARKHMKSSSLAIREMOIKTMYRHLTPVRMA	1125
AIYSSDGLISRIYNELQIVKKTNNPIKKWDRNRHPSKEDIYAARKHMKSSSLAIREMOIKTMYRHLTPVRMA	1140
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HITKSPNQRWRCGEGITLHCHWCKLVQPLKSWVRFLRDLLEIIPDPAPILLGIYPRDYSCCYKDTCTRMPIAA	1220
LPTIAKTNQPCPTWIDWIKKHHIYMEYAAIKND-EPNSFVQWNNKLETIILSKLSQ	1285
QPIIAKSWKPKCPTBWTWIDWIKKHHIYMEYAAIKND-EPNSFVQWNNKLETIILSKLSQ	1281

Fig. 2 Amino-acid sequences of large ORFs found in human (L1Hs, upper) and prosimian (L1Nc, lower) consensus L1 family sequences. The amino-acid sequences were deduced from the consensus nucleotide sequences and aligned by the program of Wilbur and Lipman³² in the GENAS system³³ to achieve maximum homology. Colons indicate amino acids identical in both sequences. Only the region homologous between the two sequences is presented. The numbers shown alongside each sequence correspond to those given for the large ORFs. The homology is 65% at the amino-acid sequence level and 71% at the nucleotide sequence level. Underlining indicates amino-acid sequences homologous to reverse transcriptases.

and a very large ORF of 927 amino-acid residues was found in L1Nc4 (Fig. 1). All the large ORFs in other members were found to use the same reading frame as the very large ORF of L1Nc4, and overlapped each other (Fig. 1). From these sequence data, a consensus nucleotide sequence for the prosimian L1 family was deduced and found to be capable of encoding a very large ORF of 1,294 amino acids. Similarly, a consensus sequence of the human L1 family was deduced from family members (the consensus sequences of human and prosimian are available on request). Just as in the case of prosimian, a very large ORF (1,280 amino acids) was identified at a position similar to that of the prosimian ORF (Fig. 1). Figure 2 shows the two ORFs of the consensus sequences. Human and prosimian large ORFs showed a homology of about 65%, and these ORFs, a large ORF in a mouse L1 family member (L1Md4)^{7,18} and a partial consensus ORF of rat L1 family¹⁹, were found to be using the same reading frame. Our results provide additional support for the idea that the L1 family is derived from a sequence(s) encoding a protein(s)⁷ and, moreover, suggest that the protein is a relatively large polypeptide which is evolutionarily conserved.

Fig. 3 Homology of amino-acid sequences between L1 consensus ORFs and RNA-dependent DNA polymerases of various origins. The amino-acid sequences were taken from Moloney murine leukaemia virus (MoMLV)³⁴, murine mammary tumour virus (MMTV)³⁵, bovine leukaemia virus (BLV)³⁶, human T-lymphotropic virus type I (HTLV-I)³⁷, Rous sarcoma virus (RSV)³⁸, caprine arthritis-encephalitis virus (CAEV)³⁵, cauliflower mosaic virus (CaMV)^{22,39}, 17.6 (ref. 40) and the mitochondrial class II introns Oxi 3-1 and Oxi 3-2 (ref. 41). Seven homologous sequence blocks shown by underlining in Fig. 2 are presented here. Amino acids that are common to at least five polymerase sequences and either of the L1 consensus ORFs, and amino acids in polymerase sequences identical to those of either of the L1 consensus ORFs are boxed. Ten invariant amino-acid residues²¹ are indicated by circles; the residues conserved in L1 ORFs are shown by filled circles. The 27 identical and chemically similar amino-acid residues reported by Toh *et al.*²² are indicated by triangles; among these, the residues conserved in L1 ORFs are shown by filled triangles (5 out of 27 amino-acid residues are not included in the region shown here). Using the mutation matrix of Schwartz and Dayhoff⁴², the significance of each homologous sequence block was estimated using a computer algorithm⁴³. The statistical test showed that the homologies of most of the sequence blocks between L1 ORFs and yeast intron-encoded ORFs are significant with a probability of $<1.3 \times 10^{-3}$ ($>3\sigma$).



To investigate the protein encoded by the progenitor of L1, we searched the Protein Sequence Database of the National Biochemical Research Foundation for amino-acid sequences homologous to ORFs in the human L1 family sequence T β G41; a relatively high homology was found between T β G41 ORF and transferrins⁹. Using the same approach, significant homology was also found between consensus ORFs and some retroviral reverse transcriptases. Alignment of the amino-acid sequences of RNA-dependent DNA polymerases of various origins was then carried out together with those of L1 family ORFs. As shown in Fig. 3, the L1 family ORF is evidently homologous to RNA-dependent DNA polymerases, particularly to that of yeast mitochondrial class II introns (Oxi3-1 and Oxi3-2), which show a high sequence homology to RNA-dependent DNA polymerases²⁰. Out of 102 amino-acid residues, 37 were identical between the consensus ORFs and Oxi3-1. Toh *et al.*²¹ found 10 invariant amino acids in 5 viral RNA-dependent DNA polymerases, and we found that 8 of these were conserved in human and prosimian consensus ORFs (Fig. 3, filled circles). Toh and his colleagues²² also noted 27 positions at which identical or chemically similar amino acids occurred in 8 RNA-dependent DNA polymerases. At 20 of the 27 positions, the consensus ORFs were found to possess amino acids common to the polymerases (Fig. 3, filled triangles). A highly conserved sequence, YXDD (one-letter notation), flanked by three hydrophobic residues was described by Toh *et al.*^{21,22}. Although L1 consensus ORFs use F (phenylalanine) instead of Y (tyrosine) in the sequence, the RNA-dependent DNA polymerase of the yeast Ty transposable element^{23,24} also uses F instead of Y, and the sequence LFVDDMI of L1 consensus ORFs is very similar to the sequence LFVDDMI of Ty polymerase. Thus, the L1 consensus ORF shows significant homology to known RNA-dependent DNA polymerases. It is probable that the protein potentially encoded by the L1 family or its progenitor is closely related to reverse transcriptases. If the protein is actually a reverse transcriptase, it is conceivable that the enzyme binds to its own messenger RNA immediately after translation to start cDNA synthesis. This is consistent with a preferential and active dispersion model for L1 family sequences. It will be of interest to test whether a protein carrying the amino-acid sequence of the consensus ORF actually possesses reverse transcriptase activity.

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Note added in proof: After submission of this paper, we found a report⁴⁴ pointing out the homology between the ORF of the mouse L1 family and the reverse transcriptases of retroviruses; the results of that study were consistent with ours.

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MATTERS ARISING

Physiological relevance of erythroid-potentiating activity or TIMP

WE note the unusual story unfolding in *Nature* concerning the molecular biology of a glycoprotein that acts as a tissue inhibitor of metalloproteinases (TIMP) and has erythroid-potentiating activity (EPA)^{1,2}. While the occurrence of two such disparate activities within one molecule would be interesting, the physiological relevance of these data should evoke some caution.

TIMP is a well described entity capable of blocking the action of vertebrate collagenases as well as numerous other neutral metalloproteinases, that is, gelatinases, proteoglycanases and type-specific collagenases. This inhibitor forms a tight ($K_i < 10^{-9}$) enzyme/inhibitor complex and appears to serve as a general tissue anti-proteinase³. TIMP is almost ubiquitous in human tissues. Connective tissue fibroblasts of multiple origins synthesize this product^{4,5} as do several cell types of haematopoietic origin including alveolar macrophages, HL-60 and platelets^{6–8}.

In vivo plasma concentrations of TIMP are on the order of 17 nM with a fairly narrow (~25%) standard deviation⁵. TIMP concentrations in amniotic fluid are even higher, averaging 68–102 nM. It is unlikely that the concentrations of this glycoprotein in tissue and bone marrow are significantly different; and these levels are at least 1,000-fold higher than the maximally stimulating concentrations of EPA (10–20 pM)⁹. Although it may be the case that *in vivo* conditions require significantly higher EPA concentrations than the *in vitro* assays, these data suggest that the marrow is saturated with respect to the activity of this modulator.

Thus, the dual role of TIMP/EPA appears incongruous in several respects. This glycoprotein is almost ubiquitous in human tissues and seems to be a common housekeeping product of numerous cell types, perhaps in keeping with its role as a protective agent of the extracellular matrix. While purified EPA demonstrates maximal activity at biologically anticipated concentrations, these are far exceeded by ambient levels of the glycoprotein. It may well be that the erythroid-potentiating activity of TIMP represents a coincidental property of this molecule. Alternatively, it might be best

to re-examine the properties of this glycoprotein using both purified natural products and a purified recombinant-derived protein.

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GASSON AND GOLDE REPLY—The description of a single glycoprotein with both protease inhibitor and erythroid-potentiating activity is indeed intriguing. Erythroid-potentiating activity was originally purified using a sensitive bioassay which measures the enhanced growth of normal human erythroid progenitors in semi-solid medium¹. The purified 28,000-dalton protein was subsequently sequenced, and oligonucleotide probes were generated and used to obtain complementary DNA clones². Both the purified natural and bio-synthetic (recombinant) proteins stimulate the growth of primitive and more mature erythroid precursors *in vitro*. In addition, the partially purified EPA and the purified biosynthetic material have direct effects on colony formation by human cell lines^{3,4}. The availability of human cell lines as homogeneous target cells has made it possible to pursue the mechanism of action of human erythroid-potentiating activity. Our recent studies using radioiodinated human EPA have demonstrated the presence of specific cell surface receptors on responsive cell lines, but not on other types of primary human cells or cell lines (unpublished observation). It seems that EPA has many of the properties of a haematopoietic growth factor, in that it stimulates primary cells,

responsive cell lines, and appears to bind to a specific cell surface receptor which could mediate these biological responses. We doubt whether hormonal potentiation of cellular growth observable at picomolar concentrations on target cells with specific binding sites could be considered “coincidental”.

The role of the EPA *in vivo* is, of course, a difficult question to address. Using the cDNA clone as a probe, as well as a sensitive radioimmunoassay to look for the synthesis of EPA messenger RNA and protein, we had arrived at similar conclusions to those reported for TIMP. In other words, EPA appears to be synthesized in many cell types; this is not necessarily unusual for haematopoietic growth factors (for example, colony-stimulating factor, CSF-1 or M-CSF, is produced by most, if not all, tissues examined in the mouse). Our estimate of the circulating EPA concentration in human serum was somewhat lower than 17 nM more in the range of 5–10 nM, although these levels are also high relative to the concentration of EPA required for a maximum growth-promoting effect *in vitro*. As we do not know the site of EPA action *in vivo* nor, as pointed out, do we know the actual concentration required for biological activity *in vivo*, it is hard to interpret these findings in a physiological framework.

Because of the availability of cell culture techniques, it has been possible to purify this erythroid growth factor. Molecular biological approaches have allowed us to produce sufficient material to investigate the effects on responsive target cells at the molecular level *in vitro* and at the organismic level *in vivo*. Ultimately it should be possible to reconcile the enzymatic inhibitory effects and the growth-promoting properties of the molecule. We feel it would be unwise at this point to regard the hormonal properties of this molecule as ‘coincidental’ or trivial.

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genic activity *in vivo* and are a requirement, in serum or serum-free culture, of cells from a variety of vascular tissues.

Surprisingly, the acidic form of the heparin-binding polypeptides also accounts for the neural extract requirement of normal and tumour rat and human prostate epithelial cell proliferation^{10,11,20-22}.

All three forms of the acidic factor, called prostatropin in this role, supported half-maximal proliferation rates for normal and tumour prostate epithelial cells at concentrations of 50 and 7 pM, respectively, in chemically-defined medium^{20,22}. These findings place at least one epithelial cell type in the target cell spectrum for the heparin-binding family of growth factors.

In summary, this growth factor family enables cell biologists to culture new cell types in defined medium. Until recombinant technologies for factor production are developed, heparin-affinity chromatography can provide purified factors from bovine brain or other tissues in quantities sufficient for commercial distribution. The complete spectrum of target cell types whose maintenance is dependent on these growth factors has not been established. The current spectrum, however, includes both cell types of the human vascular system which are central to understanding heart disease, atherosclerosis and abnormal angiogenesis, and one epithelial cell type important for studying prostate hyperplasia and adenocarcinoma. □

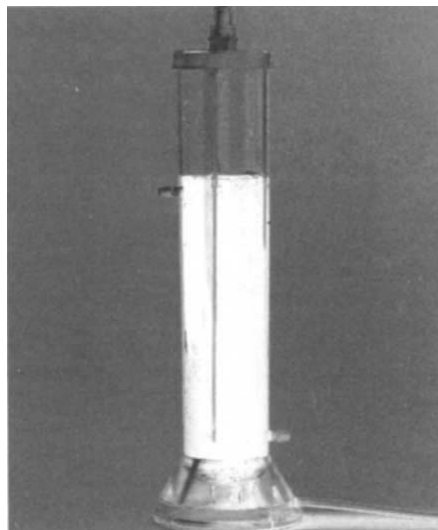
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Hi-tech for tissue culture: a growing endeavour

Persuading cells to grow outside of their native surroundings can be a complicated task, demanding elaborate materials and equipment.

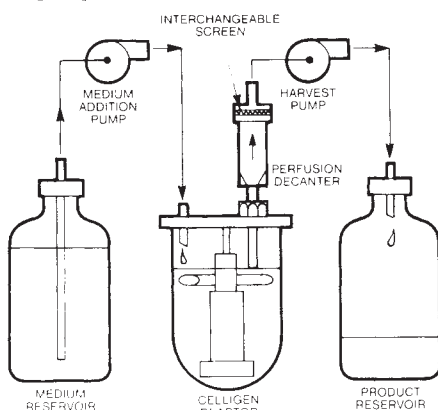
VENTREX makes a **disposable, pre-sterilized airlift fermenter** for one-time use in laboratories that don't routinely perform cell culturing (Reader Service No. 100). Their 650-ml benchtop device, called Cel-lift, can be used for batch cultures or in continuous feed mode when attached to an infusion instrument. Ventrex also



A fermenter for the infrequent user.

offers a heating jacket as an option to their system. Constructed of polystyrene, Cel-lift is suitable for bacterial as well as mammalian cell cultures, and it can maintain cultures for up to two months. Positive pressure operation reduces the risk of contamination in the fermenter. Ventrex sells a Cellift starter kit for \$225 (US); the Cel-lift unit alone costs \$125 (US).

The design of the aeration cavity on New Brunswick Scientific's **bioreactor** enables large quantities of shear-sensitive cells

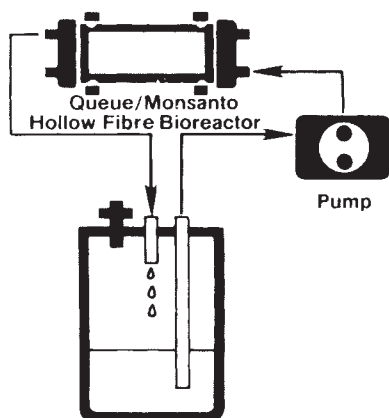


Cell perfusion is a CelliGen option.

to flourish in sparged medium without damage (Reader Service No. 101). By confining the screened cavity to a remote site on the inside of the hollow impeller, the CelliGen system protects microcarriers and suspended cells from sparging turbulence whilst freely circulating culture medium absorbs the gas. NES says that their bioreactor achieves up to 10 times the K_La efficiency of conventional surface-aeration systems that use a gas overlay. Swept-back, rotating impeller ports keep cells or microcarriers evenly dispersed throughout the CelliGen vessel, and a foam elimination chamber coalesces foam into liquid. NBS put a microprocessor in charge of the four-gas control system, which regulates the volumes of air, N₂, O₂ and CO₂ needed to support the desired dissolved oxygen saturation and pH. The microprocessor also regulates temperature to within $\pm 0.025^\circ\text{C}$, and stirrer speed to ± 1 r.p.m. over a 15- to 225-r.p.m. range. CelliGen comes in 1.5-, 2.5- and 5-litre sizes starting at \$9,450 (US), and is available with four types of impeller systems.

Bellco aims to solve similar cell culture dilemmas with a modified shape for its bioreactor (Reader Service No. 102). Their **conical glass culture reactor** requires less vigorous gas flow, thereby providing gentler circulation for suspended cells and microcarriers. Bellco claims their design also eliminates foaming in high-serum media. The 3.5-litre vessel has a sealed water jacket to reduce contamination, and a microprocessor takes care of harvest/feed cycling to minimize supervision. Bellco's reactor, with its nine-port head, can be expanded via accessory panels. The company also offers an optional dispenser that produces macrocarrier alginate gel beads for cell immobilization. With Bellco's machine, batch or perfusion cycling can be specified through programmable cartridges for the bioreactor's microprocessor.

Modelling the *in vivo* environment, a **hollow fibre perfusion bioreactor** developed by a Queue Systems—Monsanto collaboration lets nutrients flow to culture cells whilst carrying waste away (Reader Service No. 103). A pumping system and a media reservoir complete the basic reactor system, but Queue/Monsanto recommend a more elaborate control system

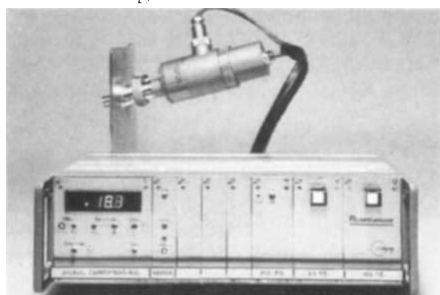


Fresh Medium Reservoir

The Hybrinet low-shear configuration.

(the Queue Mouse, in particular, was mentioned). Hybrinet is composed of biocompatible materials and does not require pre-sterilization methods or wetting agents that could confer toxicity. The Luer-Loc penetrations accommodate a variety of fittings, probes, valves and filters for specialized applications. Queue/Monsanto say they subject Hybrinet units to non-destructive integrity tests following fabrication. The instrument's specifications are geared towards the maintenance of eukaryotic cells in a low-shear environment: fibres measure 9 inches with a 300 Å pore size, and the flow rate can vary between 10 and 40 ml min⁻¹. The hollow fibre module can be purchased alone for \$375 (US), or with fittings and a support stand at additional cost.

A **fluorescence probe** from Ingold has just been introduced by Life Science Laboratories in the United Kingdom (Reader Service No. 104). The Fluorosensor can garner information for the



Fluorosensor by Life Science Laboratories can estimate biomass concentration.

control of oxygen supply and substrate feed and can be sterilized along with bioreactor vessels. Proteins, enzymes, co-enzymes, pigments and primary or secondary metabolites can all serve as fluorophores, if subjected to the correct excitation wavelengths. The £10,200 (UK) Fluorosensor fits into standard DN25 ports. Once proper excitation wavelengths have been determined for a number of biological compounds, the method's flexibility could open up new control and monitoring options for many biotechnological processes.

The first chapter of a technical support publication from LH Fermentation Ltd. gives practical advice on fermenter use in **continuous culture fermentation** (Reader Service No. 105). This issue of *Hints & Tips* also expounds upon the applications of continuous culture, as well as problems frequently encountered with this particular technique. LH Fermentation, which recently expanded their airlift fermenter range with a 10-litre version, expects to continue their *Hints & Tips* series chapter by chapter to build a complete laboratory

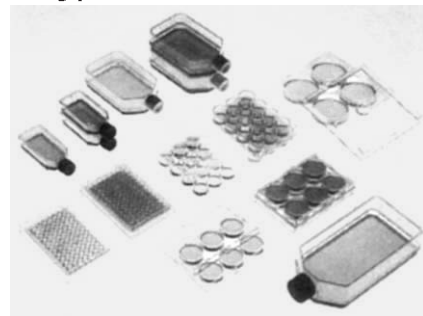


The first in a fermentation series

manual on fermentation. This first issue comes as part of a larger literature package including LH's newsletter and their 1986 price list.

Labware lookout

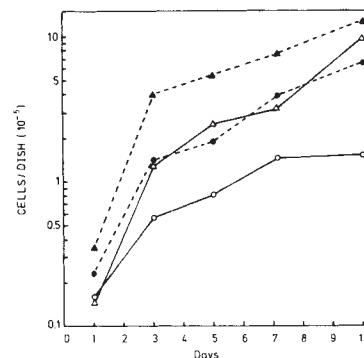
Where tissue culture is concerned, **labware coated with extracellular matrix** is often touted as the next best thing to being there. In Vitro International now produces flasks in 25-, 75- and 150-cm² sizes coated with bovine ECM, to complement their existing supply of ECM-coated 4-, 6-, 12-, 24- and 96-well plates (Reader Service No. 106). This labware, used like plastic culture labware but stored at 4°C, owes its origin to the efforts of endothelial cells, which excrete ECM into the labware during proliferation. The matrix adheres



IVI expands their ECM-coated labware selection.

strongly to the plastic, and subsequent removal of the endothelial cells leaves the substratum behind. In Vitro's flasks cost between \$20 and \$120 (US) in single units or packs of five, and have a shelf-life of four months.

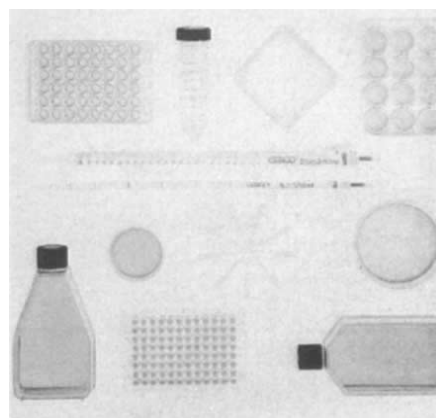
ECM cultured cell lines show reduced requirements for serum supplements and



Cell proliferation on IBT's ECM-coated plates (dotted lines) vs. traditional labware (solid lines).

cellular mitogens while at the same time exhibiting greater attachment, migration, proliferation and in some cases differentiation, than those reared in traditional plastic labware. International Bio-Technologies supplies a wide selection of ECM-coated **tissue culture plastics** and also provides custom-coating services (Reader Service No. 107). One such service is the coating of labware disposables with radiolabelled ECM, tailored to specific customer needs. IBT has compiled technical literature describing the use of their substrate in human amniotic cell and human primary malignant epithelial cell cultures. The main goal is the rapid diagnosis of prenatal disorders and perhaps the *in vitro* study of biologically derived antitumour agents.

Gibco Laboratories, familiar source of cell culture reagents, has taken the plunge into **tissue culture treated labware** (Reader Service No. 108). Gibco has

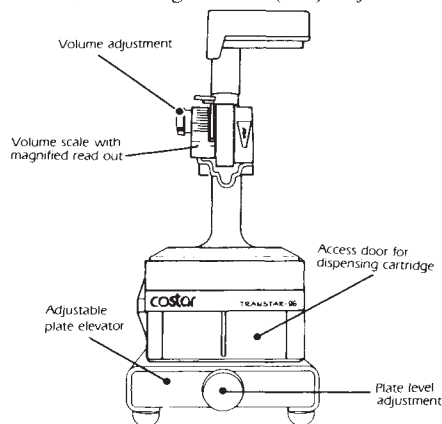


Gibco goes with surface effects.

flasks, dishes, cluster dishes and roller bottles that have been scored with electron beams to improve cell growth. The company tests its labware for growth support; adherent heteroploid cell lines including CHO-K1, BHK-21 and VERO are used in these plating efficiency and monolayer proliferation assays. Pipettes, microtitration plates, freezing vials and

centrifuge tubes are also part of Gibco's labware introduction. A 12-page catalogue gives more information on the new products.

A portable **96-tip dispenser system** from Costar has applications in changing cell culture media, sterile partial removal of media, diagnostic screening assays, and research on and isolation of pathogens (Reader Service No. 109). The 648-gram Transtar will dispense volumes between 25 and 200 μ l, under sterile conditions. The entire Transtar unit can be autoclaved, including the \$96 (US) adjustable



Transtar protects microplate and user from contamination.

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plate elevator that allows plate-to-plate transfers and removal of media without disturbing monolayers. Costar's \$496 (US) instrument has a reproducibility of between ± 5 per cent at 25 μ l volumes and ± 2 per cent with 200 μ l volumes. It comes with disposable reagent reservoirs having 8 or 12 separated compartments, or with an open reservoir.

Chemistry for culture

Techniques for **mononuclear cell separation** must welcome a newcomer: Sepratech Corp. has a system based on continuous density gradient centrifugation using their colloidal silica-based medium, Sepracell-MN (Reader Service No. 110). One to six ml of whole blood, added directly to Sepracell tubes, separates with 10 minutes of centrifugation on the gradient established by the rapid sedimentation of Sepracell-MN particles. The resulting MNC fraction segregates above a wide band of Sepracell-MN, whilst the rest of the components are sequestered beneath this band. Sepratech's technique also entails the use of a 'Seprapette' that makes band recovery simple and more complete. The procedure should only be applied to EDTA and heparin anticoagulated blood. Sepratech's \$75 (US) package includes 50 sterile tubes, wash media and 50 Seprapettes; Sepratech also gives free samples.

Nearly 500 items for tissue culture are the subject of Sigma's 1986/87 *Cell Culture Reagents Catalogue* (Reader Service No. 111). The brochure's 100 pages cover culture staples such as RPMI-1640 and fetal



A culture catalogue with terminology and methodology.

bovine serum, as well as reagents that only Sigma provides, such as cell culture tested biochemicals.

Shear sensitivity

A small volume rotor/stator from Biospec Products can homogenize tissues in less than one minute (Reader Service No. 112). The Tissue-Tearor runs at 28,000 r.p.m. with a 4-inch long probe that draws



Biospec's stainless steel tissue homogenizer.

suspended material into the stator, where the tissue gets repeatedly cycled through six narrow slits. Volumes less than 0.5 ml are fair game for Biospec's device, and samples can be kept cold during the Tissue-Tearor's operation by immersion in an ice bath. The 14-oz instrument can be hand-held or stand-mounted. Speed control allows flexibility: the \$388 (US) Tissue-Tearor will mix, emulsify, shred and chop.

Roller assemblies for the cultivation of mammalian cells give these fragile cultures gentle treatment (Reader Service No. 113). New Brunswick Scientific has two RollaCell apparatus, RC41 and RC42, that provide precise control of rotation and adhesion for shear-sensitive cells. These units can be expanded from four-place roller assemblies to seven-tiered structures, thereby facilitating mass production. Even larger-scale production models are available, with up to nine tiers and space for 500 mm roller bottles. These systems also incorporate fail-safe devices that signal problems in operation. NBS offers the RC41 and RC42 for £1,231 and £1,425 (UK) respectively.

Luckham Ltd also has a system for continuous **rolling of tissue culture vessels**, with a capacity of up to 25 bottles (Reader Service No. 114). The RMV Universal Roller features a speed meter that fixes rolling speeds up to 60 r.p.m. with a minimum of variation. Luckham's roller will



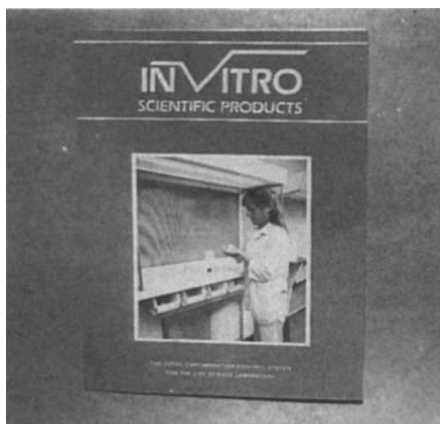
Luckham Ltd is on a roll.

accommodate as many as five tiers and the installation of extra rollers provides a configuration for smaller tubes. The RMV goes for £886 (UK).

Keeping it clean

Sterivex filter units from Millipore now come in 0.22 and 0.45 μm pore sizes for the **sterilization and clarification** of tissue culture media, additives and other biological solutions (Reader Service No. 115). Millipore's low-protein-binding, low-extractable Durapore membranes were designed to be biocompatible with cell cultures. Sterivex units obviate the need to decant into storage containers and risk re-contamination; instead, they filter directly into the final container with the aid of a pressure vessel, pump or repeating syringe. Millipore, which sells 10 Sterivex units with filling bells for \$35 (US), claims the pressure-driven design of its filters eliminates foaming.

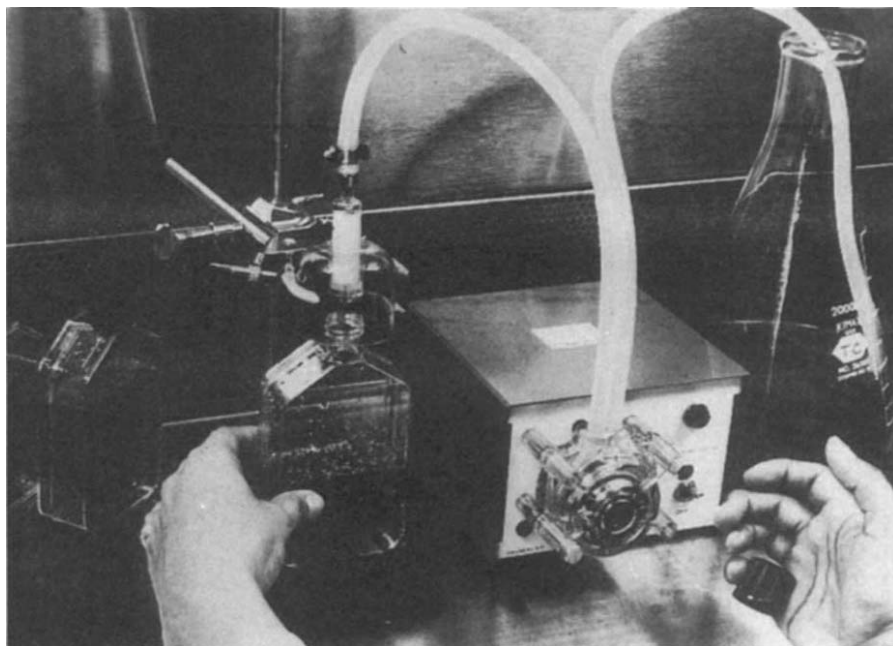
A 4-page mini-catalogue on **anti-microbial/contamination control products** may help tissue culture scientists bring con-



In Vitro's guidelines for contamination control.

tamination to a halt (Reader Service No. 116). Features in the catalogue from In Vitro address environmental sources of contamination, such as incubators, laminar flow hoods and bench tops. The brochure also presents a handwashing procedure aimed at minimizing cross-contamination from technicians' hands.

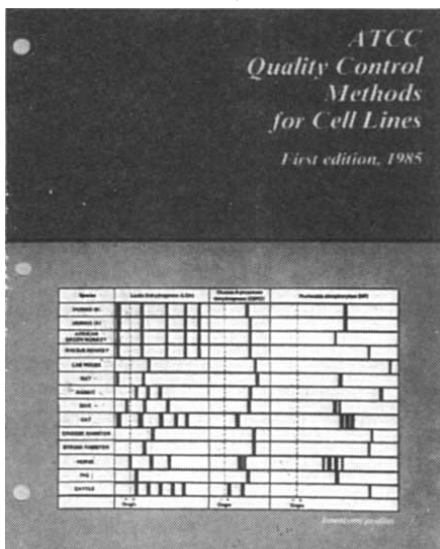
Another laboratory manual comes from the American Type Culture Collection, this one titled *ATCC Quality Control Methods for Cell Lines* (Reader Service No. 117). The 81-page booklet, which ATCC provides for \$9 (US), contains step-by-step instructions for media preparation and testing, preservation of cell cultures and characterization of immunoglobulin products. The manual also covers safety procedures and tips for establishing or verifying cell line identity by fluorescent antibody staining, isoenzymes and chromosome analysis.



Millipore makes filters for tissue culture media clarification.

Controlled environments

Precision temperature control is no easy feat in **incubator rooms** full of cell culture apparatus, roller machines and water baths, but Genetic Research Instrumentation claims to have accomplished just that (Reader Service No. 118). GRI combined a proportional voltage controller and air circulation system that uses terylene diffusers to minimize temperature gradients in their walk-in modular rooms. With this style of control, GRI says they can reach accuracy of $\pm 0.1^\circ\text{C}$. Being modular, the incubator rooms can be built to any size standard starting at 4 square feet, and take only 3 days to complete. These temperature controlled rooms are suitable for animal, plant and microbial cell growth; prices vary with size.



Microbial contamination testing and more.

Design advances on ESPEC Corp.'s **carbon dioxide incubator** side-step many of the problems such chambers can engender (Reader Service No. 119). To prevent contamination, the walls and corners have been rounded, and the shelves, shelf brackets and rear duct wall can be removed without tools, all for easy cleaning. A 'vertical down' air convection system and microprocessor-controlled heater, along with triple-walled construction, also thwart condensation build-up on chamber walls and ceiling. Because all the electronic components of ESPEC's CO_2 unit are contained on one circuit board, replacement is easy: the company guarantees board replacement within 24 hours. In the United States Tekmar sells the incubator for \$2,850.

ASTEC Environment Systems have just put a range of **laminar flow cabinets** on the market to complement an existing collection of filtration fume cupboards (Reader Service No. 120). The cabinets come with either horizontal or vertical airflow in a variety of sizes and construction materials. Astecair Biological Safety Cabinets have vertical laminar flow and conform to British guidelines for handling ACDP groups 1, 2 and 3 pathogens. These units are made from stainless steel and fitted with two HEPA filters for recirculated and exhaust air. ASTEC also manufactures Astecair Horizontal Laminar Airflow Cabinets in stainless steel or white enamel and three widths, as well as Astecair Vertical Laminar Airflow Cabinets in a small, low-cost unit or a larger high-quality model. □

These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal.

No quick cure for skill shortages

from Richard Pearson

At a time when Britain's old industries are rapidly contracting there are still skill shortages in the new ones. Government action may be essential.

THE development and application of information technology is an essential ingredient for a successful economy, but since the 1970s there have been complaints that a shortage of the appropriate skills has acted as a constraint on the development of information technology in the United Kingdom. We are not alone with this problem, skill shortages at professional level are also affecting the United States and many European countries. Information technology is big business worldwide and a major source of growth. The output of the UK industry, which includes computer and telecommunications equipment, software and computing bureaux and consultancies, is now worth over £6,000 million per annum and has been growing at 20% annually in recent years. The market has been growing even faster, however, and there was an adverse trade balance in the United Kingdom of around £1,000 million in 1985.

British companies have claimed that skill shortages have held them back. Half of UK employers in the information technology field suffered skill shortages last year, the skills most sought after including software development, programming, systems analysis and particularly experience of developing and applying 'systems'. Nearly half of the companies approached for a recent survey subcontracted information technology work, often because they were unable to attract sufficient staff with specialist skills (H. Connor & R.P., *Information Technology Manpower into the 1990s*; Institute of Manpower Studies, 1986).

There are now over 200,000 professional information technology staff employed in the United Kingdom. The industry itself is a dominant employer of these skills, many of these companies each employing more than 1,000 such people. The 'user' companies, however, often rely on their existing engineers and technologists to apply the technology with little specialized training (Fig. 1).

While the public sector and many financial organizations often train school leavers or retrain existing staff for information technology jobs, the majority of employers seek to recruit either ready-trained staff or new graduates. While problems of labour turnover and stories of 'poaching' regularly hit the headlines, particularly in London's money markets preparing for the 'big bang' this autumn, labour turnover was not seen as a problem

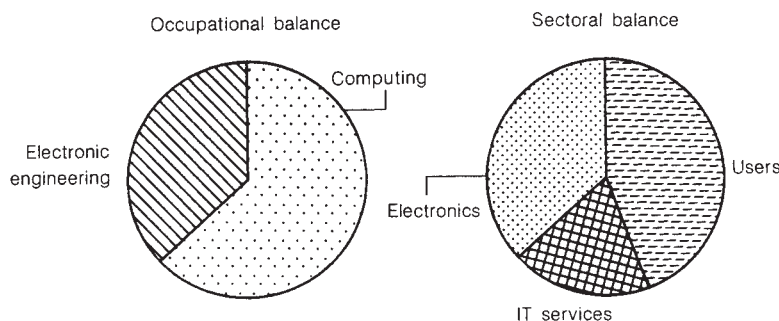


Fig. 1 Occupational balance and sectoral balance of UK information technology (IT) professionals in 1985.

for employers approached for the IMS questionnaire, many experiencing turnover rates of 5% or less. This was especially the case for electronics-based skills, with most problems occurring in the field of software and computing skills, where turnover rates were sometimes in excess of 20%. This was, however, due to many diverse factors including the imminent de-regulation in the City as institutions rush to set up computerized dealing systems, fast growing companies having large numbers of short-service staff, who inevitably have higher wastage rates, and company reorganizations where staff are not waiting to see what is in store for them, preferring to move to what appear to be more secure environments. The changing role of central data processing departments is also causing a great deal of strain. As the role of the user increases, central staff now have to develop new consultancy and advisory skills if they are not to be bypassed by user departments developing their own staff to be 'applications consultants' to advise on the purchase and installation of information technology equipment.

What then are the prospects for skill shortages to the end of the decade? With the market expected to grow by 10–20% per annum, demand for information technology professionals will clearly grow. The precise number and skills needed will be affected by many factors including economic growth, the rate of diffusion of information technology into manufacturing industry and the public sector, the changing balance between imports and exports and for the electronics sector the scale of reductions in spending on UK defence equipment. Overall the demand for information technology professionals could grow by 5% per annum and total 250,000 or more by 1990.

Higher education, which currently produces about 6,000 first-degree graduates in information technology each year, will remain the key source of newly qualified staff for information technology jobs. The demand for new graduates could grow by 50% or more over the next five years. While overall finance for higher education is being cut back there are a number of initiatives under way to boost the supply of information technology graduates, but skill shortages are likely to continue over the period to 1990, barring any major economic downturn. The biggest problem will continue to be in relation to experienced staff. For employers, improved salaries and conditions will help improve their market share of scarce skills. A greater commitment to, and investment in, training and retraining of existing staff will, however, have to be an essential form of self-help to alleviate overall shortages, despite fears of poaching, which are often over-sold. Improved utilization of existing staff and technicians will also yield beneficial results.

Finally, there is a need to support the education system to encourage girls and more students generally to study subjects relevant to information technology. Otherwise the 35% downturn in the number of 18-year-olds over the next decade will have a significant impact on the size of the pool of potential students available for information technology courses. All levels of education are also desperate for more resources. Some improvements are under way to increase skills availability, but these need to be further developed, and quickly, if skill shortages are not to remain a continuing constraint on the economy. □

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